IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: GOLDENBERG
Serial No.: 09/965,796
Filed: October 1, 2001
Title: IMMUNOTHERAPY OF B-CELL MALIGNANCIES USING ANTI-CD22 ANTIBODIES
Group Art Unit: 1643
Examiner: Alana M. Harris
Attorney Docket No.: IMMU:007US3
Confirmation No.: 3640

EFS-WEB

BRIEF ON APPEAL UNDER 37 CFR §41.37

COMMISSIONER FOR PATENTS
P.O. BOX 1450
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Sir:

This appeal brief is being filed in accordance with the provisions of 37 C.F.R. § 41.37. The fee of $255.00 under Rule 17(c) for filing of this brief is addressed in the accompanying transmittal. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to deposit account 18-2056. If any extensions of time are needed for timely acceptance of papers submitted herewith, Appellants hereby petition for such extension under 37 C.F.R. §1.136(a) and authorizes payment of any such extensions fees to Deposit Account No. 18-2056.

I. REAL PARTY IN INTEREST

The real party in interest in this application is Immunomedics, Inc., as evidenced by an assignment filed in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

A notice of appeal has been filed in SN 10/314,330, which is a continuation of the present application. A brief on appeal has not yet been filed in this other case.

III. STATUS OF CLAIMS

Pending claims: 24-27, 36-44, 47, 52, 55-59, 98 and 99

Canceled claims: 1-23, 28-35, 45, 46, 48-51, 53, 54, and 60-97

Rejected claims: 24-27, 36-44, 47, 52, 55-59, 98 and 99

Appealed claims: 24-27, 36-44, 47, 52, 55-59, 98 and 99

IV. STATUS OF AMENDMENTS

No claim amendments were filed subsequent to final rejection. All claim amendments have been entered into the record and are reflected in the appendix.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The sole independent claim on appeal is claim 24, which recites a method for treating a subject having a B-cell malignancy, comprising administering to the subject a therapeutic composition comprising a pharmaceutically acceptable carrier, and an immunoconjugate, wherein the immunoconjugate comprises

(i) at least one human, humanized or chimeric anti-CD22 antibody, and

(ii) a drug or a radioisotope,

wherein the immunoconjugate is used in combination with a naked anti-CD20 mAb.

Support for a method for treating a B-cell malignancy is found on page 1, lines 7-8. A pharmaceutically carrier is supported by the disclosure at page 26, lines 24-25. Chimeric and humanized anti-CD22 antibodies were recited in original claim 12, and disclosed at page 6, line 19. Human antibodies are described at page 10, lines 3-14. Production of chimeric, human and humanized antibodies is described in Section 3, beginning on page 7. Immunoconjugates with

drugs or radioisotopes are disclosed in Section 5, beginning on page 12. Co-administration of anti-CD22 antibodies with anti-CD20 naked antibodies is disclosed on page 23, lines 26-28.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following grounds of rejection are to be reviewed on appeal:

1. The rejection of claims 24-26, 36-38, 44, 47, 52, 55-57, 98 and 99 rejected under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and Li *et al.*

2. The rejection of claims 24-27, 36-38, 44, 52, 55-57, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and United States Patent No. 5,106,955.

3. The rejection of claims 24-26, 36-42, 44, 52, 55-57, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and United States Patent No. 5,686,072 and PCT publication WO 95/09917.

4. The rejection of claims 24-26, 36-39, 44, 45, 52, 55-57, 60-70, 73-77, 91-93, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and European Patent Application No. 510949.

5. The rejection of claims 24-27, 36-38, 43, 44, 52, 55-89, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and United States Patent No. 5,698,178.

6. The rejection of claims 24-27, 38, 43, 44, 52, 55-89, 98 and 99 under 35 U.S.C. §103(a) based on WO 96/04925 in view of Maloney *et al.* and United States Patent No. 5,698,178.

There also is a provisional rejection of claims 24-27, 36-44, 47, 52, 55-59, 98 and 99 under the judicially created doctrine of obviousness-type double patenting over claims 24-44 of SN 10/314,330, but this rejection is being held in abeyance until such time as allowable subject matter has been indicated in the second of the two cases to be allowed. MPEP 822.01. Thus, there is no need to review this rejection on appeal.

VII. ARGUMENT

A. The rejection of claims 24-26, 36-38, 44, 47, 52, 55-57, 98 and 99 rejected under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 ("Leung") in view of Maloney *et al.* ("Maloney") and Li *et al.* ("Li").

1. The art cited in the rejection.

Leung describes immunoconjugates of LL2 with cytotoxic agents or labels (see abstract). The Examiner admits that Leung does not teach combinations of LL2 with anti-CD20 antibodies as recited in claim 24 and claims dependent thereon, but urges that it would have been obvious to combine anti-CD22 immunoconjugates and naked anti-CD20 antibodies based on the disclosure in Maloney of treating B-cell lymphoma, NHL, and other leukemias and lymphomas with a chimeric anti-CD20 monoclonal antibody, rituximab. The Examiner argues that a skilled artisan would have expected a mixture of antibodies to the different epitopes "would be more efficacious in therapeutic methods, as well as enhance the treatment modality," citing the last paragraph on page 2465 of Maloney.[1] Li *et al.* is relied upon as teaching four anti-CD22 monoclonal antibodies recognize CD22 A and B epitopes, and the Examiner urges that it would have been obvious that mixture of antibodies to different epitopes of CD22 would be more efficacious.

2. Maloney does not support the obviousness of combinations of anti-CD22 immunoconjugates and anti-CD20 naked antibodies.

Maloney is the sole reference cited in support of the obviousness of combinations of anti-CD22 immunoconjugates and anti-CD20 naked antibodies. However, Maloney *teaches away* from any use of immunoconjugates, and thus is improperly combined with Leung to allege the obviousness of the presently recited combination. "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, ***would be discouraged*** from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley*, 31 U.S.P.Q.2d 1130 (Fed. Cir. 1994), emphasis added. More recently, in *Ecolochem, Inc. v. Southern California Edison Company*, 227 F.3d 1361 (Fed.Cir. 2000), cert. den., 121 S.Ct. 1607, the Federal Circuit noted that the combination of two prior art references does not render patent claims obvious if there was no evidence of any suggestion, teaching, or motivation to combine the information from the prior art and where there was evidence that the prior art actually taught away from the patented process. In *Ecolochem*, the prior art taught away from a mixed-bed

[1] Official Action dated June 1, 2007, page 5.

ion exchange process; therefore, no motivation existed for one of ordinary skill in the art to produce the patented technology.

Similarly, here Maloney teaches away from the use of antibodies that are radiolabelled or conjugated to a cytotoxic agent, noting that:

> The [anti-CD20] antibody preparation is used directly for therapy, not requiring conjugation to drugs, toxins, or radiolabels, each of which requires extensive safety testing and may not be stable after formation of the active conjugate. Antibody modification may interfere with antigen binding... significant hematologic toxicity is associated with the use of high-dose radiolabeled conjugates...In some studies, immunotoxin conjugates have been associated with significant toxicities (page 2585, penultimate paragraph).

Thus, a skilled artisan ***would be discouraged*** from the very combination urged to have been obvious by the Examiner. The combination of Leung and Maloney would not have suggested therapy with a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody. No *prima facie* case of obviousness of claim 24 and claims dependent thereon is supportable based upon the combination of a primary reference that teaches the use of immunoconjugates (Leung) and a secondary reference (Maloney) that teaches the use of naked antibodies and specifically teaches away from any use of immunoconjugates. Li *et al.* is relied upon only as teaching a mixture of antibodies to different epitopes of CD22 would be more efficacious, and does not overcome the failure of Leung and Maloney to suggest a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody.

3. Conventional therapies, circa 1994, were chemotherapies, not antibody therapies.

The cited portion of Maloney discloses that "extension of these studies to patients with minimal disease, using antibody alone or in combination with ***conventional therapies***, may provide the greatest benefit. "Conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies. Therefore, the disclosure in Maloney that anti-CD20 may be combined with a "conventional therapy" would not have suggested a combination with anti-CD22 immunoconjugate therapy, as presently claimed. No *prima facie* case of obviousness exists.

The Examiner commented that "anti-CD22 is regarded as a conventional therapy" and that Maloney notes that "using antibody [CD20] alone or in combination with conventional therapies,

may provide the greatest benefit." The Examiner cites Webster's Collegiate Dictionary as defining "conventional" as meaning "developed, established, or approved by general usage; customary." She then urges that Leung makes it clear that therapy with anti-CD22 antibody "has been developed and established and is reasonable regarded as a conventional therapy as supported by the definition of 'conventional.'" In this regard, she notes that references directed to anti-CD22 therapies have dates as early as 1991.

Maloney suggests the possible implementation of further studies in which the CD20 antibody is combined with "conventional therapies." If the Examiner is urging that all antibody therapy was "conventional" as of 1994, such that a skilled artisan would have been motivated to combine a different antibody, such as an anti-CD22 antibody, in treatment, based on Maloney's comment regarding the addition of "conventional therapies" to his anti-CD20 antibody, this is unsupported by anything in the record. Certainly Maloney itself does not support the Examiner's statement that treatment with an antibody was "conventional therapy" in 1994. Maloney is a report of results from a ***Phase I clinical trial*** to evaluate the safety of anti-CD20 antibody as a single agent therapeutic. A Phase I trial is the earliest stage in clinical trials of an ***investigational drug*** – and therefore Maloney is antithetical to the Examiner's conclusion that treatment with an antibody constituted "conventional therapy." "Conventional" means "conforming to established practice or accepted standards; traditional" (The American Heritage® Dictionary of the English Language: Fourth Edition - 2000). An investigational drug in Phase I clinical trials cannot be considered a conventional therapy, *i.e.*, it does ***not*** conform to established practice or accepted standards." By definition, investigational drugs have not been "accepted." Companies can provide investigational drugs to doctors if they are part of a drug trial covered by an FDA-approved protocol, and such drugs are ***by definition <u>not</u> conventional***, since they are not available for use by any doctor on any patient.

The first approved antibody for therapy of any malignancy was the anti-CD20 antibody rituximab that is the subject of Maloney, but it was not approved until 1997, and therefore there was no cancer therapy with any antibody that was a conventional therapy in 1994, let alone a combination therapy with multiple antibodies. Even today, after the advantage of epratuzumab combined with rituximab has been published (see below), this combination has not been approved and hence is ***not*** conventional therapy. In fact, ***no antibody combination has ever been approved***.

4. Publications support the fact that antibody therapy, and more particularly combination antibody therapy, is not conventional.

Current reviews and texts support the fact that antibody therapy, and more especially combination antibody therapy, is not conventional. Some articles began to discuss the possibility of such combination therapies following Appellants' publication of their studies of epratuzumab and rituximab in about 2002/2003, but none indicate that such therapy is "conventional." The following articles show that antibody therapy generally, and combination antibody therapy in particular is not considered "conventional" in the art, even today:

- Hiddemann in 1995 states that "more experimental approaches consist of the application of immunotoxins or radioisotopes, coupled to monoclonal antibodies directed against lymphoma-specific antigen" for the treatment of NHL, *i.e.*, even single antibody therapy was considered an "experimental approach and not a conventional therapy. *Eur. J. Cancer*, 31A(13-14):2141-5 (1995).

- Skarin *et al.* in 1997 alludes to "the use of specific monoclonal antibodies directed against cell surface antigens has contributed to the understanding of [NHL]." That is, the antibodies were considered useful in understanding NHL, but not in therapy. With respect to therapy, Skarin *et al.* list "combination chemotherapy without or without regional radiotherapy." *CA Cancer J. Clin.*, 47(6):351-72 (1997).

- An educational review published in 1998 notes under the heading "Monoclonal Antibodies" that:

  > New treatment approaches for low-grade lymphoma include monoclonal antibodies that attach to receptors found on B-lymphocytes. One general approach uses radiolabeled antibodies; another uses a "naked" antibody. *Preliminary studies of these monoclonal antibodies as single agents* has demonstrated encouraging response rates and some evidence of long-term disease control, but the median duration of response and impact on overall survival are still unknown.
  >
  > Webster *et al.*, *Oncology*, 12(5):697-714 (1998) -- emphasis added.

- In 1999, Czuczman *et al.* showed that a combination treatment of anti-CD20 monoclonal antibody and CHOP chemotherapy (a "conventional therapy") showed

improved efficacy. This is a treatment of anti-CD20 antibody and conventional therapy as mentioned in Maloney, but does not suggest combination antibody therapy. *Journal of Clinical Oncology*, 17:268, (1999).

- In an article about the current therapeutic paradigm for the treatment of NHL, Fisher in 2000 noted that patients with indolent NHL may be treated with single-agent alkylating agents, radiation therapy, or combination chemotherapy, while indicating that none of these approaches have produced curative results. Fisher notes the need for "innovative treatment strategies," and mentions the use of interferon, monoclonal antibodies with or without radioisotopes, purine analogues, and even high-dose therapy with stem-cell rescue are under investigation. Thus, in 2000, each of these was still considered investigational, and combinations of antibodies are not suggested. *Semin Oncol.* Dec; 27(6 Suppl 12):2-8 (2000).

- "What is New in Lymphoma," published in 2004, cites rituximab as an advancement in the treatment of NHL. Efforts to improve the activity of rituximab are noted, and include increasing the number of weekly infusions, delivering higher doses and increasing dose density. Combinations with CHOP are also mentioned. A Phase II study of a combination of rituximab with epratuzumab reported in 2003 and a phase I/II study of the combination of galizimab are mentioned, demonstrating that combination antibody therapy was still very much investigational at this later date. Cheson, *CA Cancer J Clin.* Sep-Oct; 54(5):260-72 (2004)

- The 2003 Merck Manual lists "many new treatments ... for indolent lymphomas. These include monoclonal antibodies, which bind to lymphoma cells and kill them. These antibodies (immunoglobulins), such as rituximab, are given intravenously. Sometimes, the monoclonal antibodies are modified so that they can carry radioactive particles or toxic chemicals directly to the cancer cells in different parts of the body. It remains uncertain whether these monoclonal antibodies can cure non-Hodgkin's lymphomas, or if they can achieve better results when combined with chemotherapy. Combinations with other antibodies are not included in the list of conventional or new therapies.

Indeed, even later articles published by IDEC fail to suggest combinations of their anti-CD20 antibody with other antibodies. In 2001, they published "Non-Hodgkin's lymphoma: review of conventional treatments" (*Curr Pharm Biotechnol.*, 2(4):279-91 (2001)), which states that:

> Conventional treatment for patients with newly-diagnosed non-Hodgkin's lymphoma (NHL) includes radiation or chemotherapy. In addition, those with asymptomatic low-grade disease may follow a "watch and wait" approach. Single agent oral alkylating therapy and CVP (cyclophosphamide, vincristine, and prednisone) have become a mainstay of treatment for low-grade NHL. High intensity chemotherapy consisting of the anthracycline, doxorubicin along with cyclophosphamide, vincristine and prednisone (CHOP) is offered as standard treatment for intermediate-grade NHL... Novel approaches to treatment are therefore needed. Monoclonal antibodies may fulfill this need, administered either as single agents or in conjunction with conventional cytotoxic approaches.

And in 2000, Maloney himself published "Monoclonal antibodies in lymphoid neoplasia: principles for optimal combined therapy," (*Semin Hematol*, 37(4 Suppl 7):17-26 (2000)), which speaks of the possibility of trying "novel combinations" and suggests that randomized, prospective trials are required to determine clinical utility. Thus, statements in later IDEC articles, including one by Maloney, contravene the Examiner's position that Maloney's mention in 1994 of a combination of anti-CD20 antibody with "conventional therapies" would encompass combination antibody therapy as presently claimed.

5. Well-known experts in the field of antibody therapy have attested that antibody therapies were not "conventional" circa 1994, and this evidence is unrebutted in the record.

Appellants have made of record three declarations under 37 CFR §1.132 by three well-known experts in the field of antibody therapy. Dr. Foon has known Dr. Goldenberg of Immunomedics and the Garden State Cancer Center for many years as a researcher in the field, and they interact at meetings. Dr. Foon also visited with Dr. Goldenberg at the Garden State Cancer Center in New Jersey on one occasion about 5 or so years ago. Dr. Leonard is a long-term colleague of Dr. Goldenberg, and has co-authored several clinical papers involving epratuzumab with Dr. Goldenberg. Dr. Leonard also has been principal investigator on three studies for Immunomedics, and has received some small consulting fees for time spent with interested companies reviewing Immunomedics' products. A few years ago, Dr. Czuczman's research group became a subcontractor on a grant awarded to Garden State Cancer Center, where Dr. David

Goldenberg is Program Director. The grant involved laboratory studies on antibody therapies of lymphomas. Dr. Czuczman also published an article with Dr. Goldenberg in 2003 on radioimmunotherapy of non-Hodgkin's lymphoma with ^{90}Y-DOTA humanized anti-CD22 IgG (^{90}Y-Epratuzumab), when they collaborated on a GSCC clinical trial, funded by the National Cancer Institute.

The declarations all show that antibody therapies were not "conventional" in 1994, when Maloney published the cited article. **This evidence stands unrebutted in the record.**

Dr. Kenneth Foon is the Director of Clinical Investigation and Program Director for the Leukemia and Lymphoma Program at the University of Pittsburgh Cancer Institute. He has an extensive background in the field of immunotherapy for cancer treatment, and has been the principal investigator on clinical trials relating to immunotherapy of various B-cell malignancies, including a current Phase II clinical trial to study effects of a combination of rituximab, fludarabine and cyclophosphamide on patients with previously untreated chronic lymphocytic leukemia. Dr. Foon attests that "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies. He further notes that the first approved antibody for therapy of any malignancy was the anti-CD20 antibody rituximab that is the subject of Maloney, but that it was not approved until 1997, and therefore there was no cancer therapy with any antibody that was a conventional therapy in 1994. Thus, he attests that the disclosure in Maloney that anti-CD20 may be combined with a "conventional therapy" would not have suggested to him therapy with a combination of an anti-CD20 antibody and another antibody, such as an anti-CD22 antibody. He cites his current clinical trial which is referenced in paragraph 1 of his declaration as an example of a combination of anti-CD20 (rituximab) therapy with a conventional drug therapy (fludarabine and cyclophosphamide), such as is envisioned by Maloney 1994. Dr. Foon further declares that treatment with anti-CD22 antibody also was not conventional circa 1994, and notes that Goldenberg *et al.*, *J. Clin. Oncol.*, 9: 548-564 (1991) provided results from a pilot Phase I study involving a small number of patients to see the feasibility of giving this radiolabeled antibody, involving targeting tumor and organs, doses delivered to tumor and normal organs, and any evidence of efficacy in a small number of patients, and does not establish that treatment with anti-CD22 antibody was "conventional." He cites the Cheson article, which references 2003 reports of both a Phase II study of a combination of rituximab with epratuzumab and a phase I/II study of the combination of

galiximab and rituximab, as demonstrating that combination antibody therapy was still very much investigational at this later date.

Dr. Leonard is the Clinical Director at the Cornell Center for Lymphoma and Myeloma at the New York-Presbyterian Hospital. He also has an extensive background in the field of immunotherapy for cancer treatment. He has been a key investigator on clinical trials relating to immunotherapy of various B-cell malignancies, particularly with rituximab. Currently he is the principal investigator for a phase II trial that is studying rituximab versus lenalidomide versus rituximab + lenalidomide in recurrent follicular Non-Hodgkin Lymphoma (NHL) after relapse from a rituximab-containing combination regimen. Dr. Leonard agrees with Dr. Foon that "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies, and that the first approved antibody for therapy of any malignancy was the anti-CD20 antibody rituximab (the subject of Maloney) which was not approved until 1997. Thus, he attests that there was no cancer therapy with any antibody that was a conventional therapy in 1994, and that the disclosure in Maloney that anti-CD20 may be combined with a "conventional therapy" would not have suggested to him therapy with a combination of an anti-CD20 antibody and another antibody, such as an anti-CD22 antibody. In this regard, he mentions that he currently is the principal investigator of a phase II study of combination antibody therapy, in this case rituximab plus galiximab (anti-CD80) (currently in press for publication in *Annals of Oncology*), but confirms that he would not have understood Maloney 1994 to have suggested such a combination antibody therapy based on the statement in the article that "extension of these studies to patients with minimal disease, using antibody alone or in combination with ***conventional therapies***, may provide the greatest benefit." Rather, he would have understood Maloney's statement to suggest combinations of the anti-CD20 antibody with chemotherapy, which was "conventional" in 1994. This is so because even single antibody therapy was not conventional in 1994. He echoes Dr. Foon's citation of the Cheson article, as demonstrating that combination antibody therapy was still very much investigational circa 2003.

Dr. Czuczman is an Associate Professor of Medicine at the Roswell Park Cancer Institute, Buffalo, New York, and also has an extensive background in the field of immunotherapy for cancer treatment. He has been a key investigator on clinical trials relating to immunotherapy of various B-cell malignancies, particularly with rituximab. Currently he is the principal investigator for a Phase II trial studying the effects of giving rituximab together with liposomal doxorubicin to patients with

relapsed or refractory B-cell non-Hodgkin's lymphoma, and also for a Phase III Trial of CHOP plus rituximab versus CHOP plus iodine-131-labeled monoclonal anti-B1 antibody (tositumomab) for treatment of newly diagnosed follicular Non-Hodgkin's Lymphomas. He also is the principal investigator for a phase II trial studying of the effects of administering rituximab together with galiximab to patients with stage II, stage III, or stage IV non-Hodgkin's lymphoma. Dr. Czuczman attests that the disclosure in Maloney that anti-CD20 may be combined with a "conventional therapy" would not have suggested to him therapy with a combination of an anti-CD20 antibody and another antibody, such as an anti-CD22 antibody, because "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies. He notes that two of his current clinical trials referenced in paragraph 1 of his declaration relate to combinations of the anti-CD20 antibody rituximab with a conventional drug therapy (liposomal doxorubicin or CHOP) along the lines envisioned by Maloney 1994.

Dr. Czuczman published a report in 1999, discussed above by Appellants, which showed that a combination treatment of anti-CD20 monoclonal antibody and CHOP chemotherapy (a "conventional therapy") showed improved efficacy. Czuczman *et al.*, *Journal of Clinical Oncology*, 17:268 (1999). He cites this as a treatment of anti-CD20 antibody and conventional therapy as mentioned in Maloney, and states that it also does not suggest combination antibody therapy. He also cites the Cheson article as demonstrating that combination antibody therapy was still very much investigational circa 2003.

6. The facts of *In re Kerkhoven* clearly can be distinguished from the facts of the present case.

Following submission the Rule 132 declarations, the Examiner shifted her rejection slightly, and cited the case of *In re Kerkhoven*, 626 F.2d 846 (CCPA 1980). However, the rejection still relied on Maloney as the basis for suggesting a combination of antibodies. While the Examiner no longer made statements in her Action relating to "conventional therapies," this still formed the basis of the rejection. This is clear, inasmuch as the Examiner continued to cite the last paragraph on page 2465 of Maloney, which is the paragraph mentioning the use of anti-CD20 "in combination with conventional therapies." The shift by the Examiner was in response to appellants' submitted Rule 132 Declarations from experts in the field that "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies. As noted above, this evidence stands unrebutted on the record. Hence the present rejection is improper on this basis

alone, as there the Examiner has not supported her position that therapy using a combination of antibodies would have been obvious.

Moreover, the facts of *In re Kerkhoven* clearly can be distinguished from the claims presently at issue. *Kerkhoven* is cited for its statement that "it is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).

At issue in *Kerkhoven* were claims to a process of preparing a spray-dried detergent by mixing together two conventional spray-dried detergents. The combination was held to be *prima facie* obvious. *Kerkhoven* related to the combination of two spray-dried detergents, and was concerned with obtaining an end product with good flow characteristics. To this end, separate slurries of two detergents were treated in a spray drying apparatus. This was an improvement over prior art methods in which two detergents were mixed together in one slurry and then spray dried together. The Board and the CCPA found the broad claim in *Kerkhoven* to have been obvious over the prior art process. More particularly, the Board noted that "one skilled in the art, knowing that individual detergents or certain mixtures of detergents produce particles having good free-flowing characteristics, would understand that the detergents desired in the final composition may be dried separately and then mixed."

The focus in Kerkhoven's application was a solution to problems with the flowing and storage properties of powdered detergents that contained two detergent ingredients, *i.e.*, with physical properties of the mixture. It is straightforward to predict the flowability property of combining two spray-dried detergents. There was nothing to suggest any difference in the ability of the resulting detergent in cleaning ability, a property in which some unpredictablilty might inhere. Indeed, as noted by Judges Miller and Markey, "the uncertainty and unpredictability often associated with the chemical arts is not present here." These are products that are used outside the body, and detergents that are not spray-dried have been combined for years, as noted in the background of Kerkhoven's application. Thus, the Board and the CCPA were justified in finding the

end result, a free-flowing combination of detergents, to have been obvious, although the CCPA did find one of Kerkhoven's claims to recite unobvious subject matter.[2]

The claims at issue in this case present a much different situation. In biology and chemistry, two separate agents can be antagonistic, agonistic, additive, or synergistic, and no one can predict the result until it is tried. In fact, MPEP 2144.06, which is cited by the Examiner, cautions that even the obviousness of a combination of compositions taught by the prior art to be useful for the same purpose does not always hold, citing *In re Geiger*, 815 F.2d 686, 2 USPQ2d 1276 (Fed. Cir. 1987) ("Based upon the prior art and the fact that each of the three components of the composition used in the claimed method is conventionally employed in the art for treating cooling water systems, the board held that it would have been *prima facie* obvious, within the meaning of 35 U.S.C. 103, to employ these components in combination for their known functions and to optimize the amount of each additive.... Appellant argues... hindsight reconstruction or at best... 'obvious to try'.... We agree with appellant."). Thus, it is apparent that the Office cautions examiners to determine whether it is obvious to combine two compositions on a case-by-case basis.

In the present case, a combination of antibodies is claimed: a conjugate of a human, humanized or chimeric anti-CD22 antibody, and a naked anti-CD20 mAb. The result of combining two different antibodies is not predictable. Often it is not known whether an antibody is agonistic or antagonistic, leading to unpredictability as to the result of a combination. Moreover, different antibodies may occupy nearby positions on the cell membrane and compete for sites so that no additive effect is seen. Also, when combining two antibodies it is not known whether the toxicities are additive, which would lead to an unacceptable level of toxicity. Thus, a skilled person would not have been able to predict whether a combination of antibodies would have an additive or synergistic effect or whether the antibodies would interfere with each other so that the combination would have a reduced effect. It was also a possibility that since both antibodies affect normal B-cells, they could act in concert to be more toxic than either alone.

[2] This claim issued in US 4,274,974.

7. Several published papers document an unpredictable increase in efficacy when the combination of antibodies according to the present invention is administered, with no adverse toxic effect.

Surprisingly, Appellants have discovered that a combination of anti-CD22 and anti-CD20 antibodies as presently claimed produces an unpredictable increase in efficacy with no adverse toxic effect. This unexpected result has been documented in published papers.

Leonard *et al.* disclose a combination therapy using epratuzumab, an anti-CD22 antibody, with rituximab, an anti-CD20 antibody. *J. Clin. Oncol.*, 23(22): 5044-5051 (2005), copy appended. The results achieved with each antibody separately are compared to the results with the combination. On page 5048, column 1, it is reported that the objective response (OR) rate of rituximab in patients with indolent non-Hodgkin's lymphoma (NHL) is 48%, with 6% having a complete response (CR). In the passage bridging pages 5048, column 2 to page 5049, column 1, treatment with epratuzumab is discussed. Here, 43% of patients with follicular NHL and 15% of patients with diffuse large B-cell lymphoma achieved an objective response.

The results for the combination of rituximab and epratuzumab are given on pages 5046-5047. The objective response was 63% in patients with NHL, with 56% of patients achieving a complete response. The level of complete responses is therefore significantly higher than for rituximab alone. Furthermore, in the passage headed "Response to Treatment," the authors explain that the median duration of remission for indolent and aggressive NHL had not yet been reached.

In the second and third paragraphs of column 1 on page 5049, the authors explain that the combination of epratuzumab and rituximab may be more efficacious than either antibody used alone. The authors also report that the toxicity to the combination antibody regimen was similar in nature and degree to that previously reported with rituximab monotherapy. This is quite surprising, as it might have been expected that toxicity would be greater for a combination than from a single antibody.

Thus, Leonard *et al.* teach that the combination therapy is more efficacious than either monotherapy, but that toxicity is similar in nature and degree. Particularly noteworthy is the passage bridging columns 1 and 2 of page 5049, which reports CR rates for patients with recurrent NHL of 56% for indolent follicular NHL and 42% for DLBCL, which is higher than the rates reported for rituximab alone in comparable dosing schedules (about 20%).

Stein *et al.* disclose results with another anti-CD20 antibody, IMMU-106, in combination with epratuzumab. *Clin. Cancer Res.*, 10:2868-2878 (2004), copy appended. The activity of this antibody is compared with that of rituximab and with a combination of IMMU-006 and rituximab. Figure 8 relates to an *in vitro* assay measuring the anti-proliferative effects of IMMU-106, epratuzumab, and the combination of IMMU-006 and epratuzumab. Figure 8 shows that IMMU-106 alone caused a 53% inhibition of proliferation, epratuzumab had no effect and the combination caused an 83% inhibition of proliferation. This shows that the combination has a synergistic effect which clearly could not have been predicted based on the activity of either antibody alone. Figure 9 shows the results of *in vivo* experiments in mice and shows that the combination prolongs survival.

On page 2876, column 2, third paragraph, the authors state that the combination is "synergistic." More particularly, the authors suggest that the increased efficacy may be due to "additive or synergistic effects on signaling events initiated by the anti-CD20 and anti-CD22 MAbs, although there may be little or no antiproliferative effects of the anti-CD22 MAb when given alone."

The results in Leonard and Stein demonstrate that a combination of anti-CD20 and anti-CD22 antibodies produces an increase in efficacy that could not have been predicted. By extension, this means that a toxic agent conjugated to one of the two antibodies can be affected by the administration of the other antibody as a naked antibody. That is, the present invention goes beyond the complementation of targeting two different antigens, with the naked antibody actually affecting the uptake of the antibody bearing the toxic agent. The results show that prior administration of one antibody, *e.g.*, epratuzumab, actually upregulates the expression and activity of rituximab or IMMU-106, as detailed by Stein (2004) and further elucidated in the Discussion section of Carnahan *et al.*, *Mol. Immunology*, 44:1331-1341 (2007), a copy of which is appended. The converse also is true: treatment with a CD20 naked antibody upregulates expression of CD22 and thereby increases the uptake of conjugated anti-CD22 antibody.

It was particularly unexpected that combinations of anti-CD20 and anti-CD22 immunoconjugates would be more effective when administered in combination, either concurrently or sequentially, because the anti-CD22 antibody internalizes very rapidly. See, *e.g.*, Carnahan *et al.*, *Clin. Cancer Res.*, 9:3982s-3990s (2003), a copy of which is appended. Therefore, the duration of the antibody on the cell surface and its antitumor activity may be of a very brief nature. However, when used to deliver a therapeutic, such as a conjugated radionuclide or drug, its action is not

dependent on the naked antibody, but rather upon internalization of the therapeutic. By combining the killing action of the anti-CD20 antibody which can upregulate the CD22 antigen expression and also decrease the number of lymphoma cells available for killing with the anti-CD22 immunoconjugate, an increased therapeutic efficacy is achieved. This has not been suggested in prior publications on uses of either conjugated or unconjugated anti-lymphoma antibodies. In studies with radiolabeled anti-CD20 antibodies, for example, predosing with a naked version of the same anti-CD20 antibody has been used in order to saturate CD20 antigen sites on normal tissues, thus enhancing targeting and uptake in tumor of the radiolabeled anti-CD20 antibody. This is the mechanism employed by Bexxar, a regimen employing tositumomab and Iodine I^{131} tositumomab. There has been, however, no suggestion of predosing with anti-CD20 antibody prior to giving a radiolabeled anti-CD22 antibody.

With respect to the articles submitted by Appellants, the Examiner comments that "the papers submitted by Applicants all set forth epratuzumab as the CD22 antibody used in combination with CD20 antibody, rituximab or IMMU-106. The prior art teaches LL2 monoclonal antibody, which is distinct from the CD22 antibody listed in Applicant's supporting references…arguments based on these papers are not commensurate." "LL2 monoclonal antibody" is the term generally used to refer to the murine monoclonal antibody previously known as EPB2 (see background of U.S. 5,789,554, the "prior art" referenced by the Examiner). U.S. 5,789,554, on the other hand, focuses on chimerized and humanized LL2 antibodies denoted cLL2 (mouse/human chimeric mAb) and hLL2 (humanized mAb), respectively, which include the CDRs of murine monoclonal antibody LL2. The statement that the prior art teaches LL2 monoclonal antibody, which is distinct from the CD22 antibody listed in Appellants' supporting references is inaccurate. The prior art teaches cLL2 and hLL2, and mentions LL2 in its background and as a source of the CDRs for the cLL2 and hLL2.

Epratuzumab is humanized LL2 (hLL2). Accordingly, the proffered articles which show the unexpected results that are achieved with the present combination of antibodies are a comparison to "the prior art" and are not a comparison to an antibody that is "distinct" from the antibody of Appellants' claims. The Examiner has not provided any rationale why unexpected results achieved with a combination employing hLL2 would not also extend to the chimeric LL2 antibodies of U.S. 5,789,554. Nor has the Examiner explained why the results are not commensurate in scope with a claim that recites anti-CD22 antibodies.

The comparison provided by the articles is a comparison to the claimed and more favored embodiment in U.S. 5,789,554, *i.e.*, the humanized antibody as opposed to the chimeric antibody. It is thus a comparison that is commensurate in scope with both the alleged *prima facie* case and the present claims.

8. The Examiner has not separately addressed claim 37, which recites that the anti-CD22 antibody is humanized, for which an additional basis for patentability exists.

Claim 37 recites that the anti-CD22 immunoconjugate uses a humanized anti-CD22 antibody. This antibody claimed in Leung is a humanized anti-CD22 antibody, and the antibody reported in the articles by Leonard, Stein and Carnahan, discussed above, also is a humanized antibody. Therefore, an additional basis for patentability exists for the use of combinations that employ a humanized anti-CD22, based on the foregoing discussion.

9. The Examiner has not separately addressed claim 44, which recites that the anti-CD22 antibody is hLL2, for which an additional basis for patentability exists.

Claim 44 recites that the anti-CD22 immunoconjugate uses the hLL2 antibody. This is the same antibody claimed in Leung and reported in the articles by Leonard, Stein and Carnahan, discussed above. Therefore, an additional basis for patentability exists for hLL2, based on the foregoing discussion.

B. The rejection of claims 24-27, 36-38, 44, 52, 55-57, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and United States Patent No. 5,106,955 ("Endo").

Leung describes immunoconjugates of LL2 with cytotoxic agents or labels (see abstract). The Examiner admits that Leung does not teach combinations of LL2 with anti-CD20 antibodies as recited in claim 24 and claims dependent thereon, but urges that it would have been obvious to combine anti-CD22 immunoconjugates and naked anti-CD20 antibodies based on the disclosure in Maloney of treating B-cell lymphoma, NHL, and other leukemias and lymphomas with a chimeric anti-CD20 monoclonal antibody, rituximab. The Examiner argues that a skilled artisan would have expected a mixture of antibodies to the different epitopes "would be more efficacious in therapeutic methods, as well as enhance the treatment modality," citing the last paragraph on page 2465 of Maloney. U.S. 5,106,955 is relied upon as teaching specific chemotherapeutic drugs, and the

Examiner urges that it would have been obvious to administer a therapeutic combination of a known anticancer antibody with other anticancer molecules.

The remarks with respect to Leung and Maloney apply equally here and are incorporated by reference. Endo is relied upon only as suggesting immunoconjugates with chemotherapeutic drugs, and does not overcome the failure of Leung and Maloney to suggest a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody. No *prima facie* case of obviousness exists with respect to the combination of Leung, Maloney and Endo.

C. The rejection of claims 24-26, 36-42, 44, 52, and 55-57, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and United States Patent No. 5,686,072 ("Uhr") and PCT publication WO 95/09917 ("Morrison").

Leung describes immunoconjugates of LL2 with cytotoxic agents or labels (see abstract). The Examiner admits that Leung does not teach combinations of LL2 with anti-CD20 antibodies as recited in claim 24 and claims dependent thereon, but urges that it would have been obvious to combine anti-CD22 immunoconjugates and naked anti-CD20 antibodies based on the disclosure in Maloney of treating B-cell lymphoma, NHL, and other leukemias and lymphomas with a chimeric anti-CD20 monoclonal antibody, rituximab. The Examiner argues that a skilled artisan would have expected a mixture of antibodies to the different epitopes "would be more efficacious in therapeutic methods, as well as enhance the treatment modality," citing the last paragraph on page 2465 of Maloney. U.S. 5,686,072 is relied upon as teaching administration of an unconjugated anti-CD19 antibody. PCT publication WO 95/09917 is relied upon as teaching that recombinant bispecific tetravalent antibodies are useful in both therapeutic and immunodiagnostic application, and the Examiner urges that it would have been obvious to produce a tetravalent construct comprising anti-CD22 antibodies, as well as trivalent and quintavalent fusion proteins.

The remarks with respect to Leung and Maloney apply equally here and are incorporated by reference. Uhr is relied upon only as suggesting combinations with an anti-CD19 antibody, and Morrison is relied upon only as suggesting a tetravalent construct comprising anti-CD22 antibodies, as well as trivalent and quintavalent fusion proteins. Neither document overcomes the failure of Leung and Maloney to suggest a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody. No *prima facie* case of obviousness exists with respect to the combination of Leung, Maloney, Uhr and Morrison.

D. The rejection of claims 24-26, 36-39, 44, 45, 52, 55-57, 60-70, 73-77, 91-93, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and European Patent Application No. 510949 ("Pouletty").

Leung describes immunoconjugates of LL2 with cytotoxic agents or labels (see abstract). The Examiner admits that Leung does not teach combinations of LL2 with anti-CD20 antibodies as recited in claim 24 and claims dependent thereon, but urges that it would have been obvious to combine anti-CD22 immunoconjugates and naked anti-CD20 antibodies based on the disclosure in Maloney of treating B-cell lymphoma, NHL, and other leukemias and lymphomas with a chimeric anti-CD20 monoclonal antibody, rituximab. The Examiner argues that a skilled artisan would have expected a mixture of antibodies to the different epitopes "would be more efficacious in therapeutic methods, as well as enhance the treatment modality," citing the last paragraph on page 2465 of Maloney. European Patent Application No. 510949 is relied upon as teaching conjugate formulas comprising two moieties, wherein both have physiological activity, and the Examiner urges that it would have been obvious to combine efficacious anti-tumor agents with an anti-cancer therapeutic composition.

The remarks with respect to Leung and Maloney apply equally here and are incorporated by reference. Pouletty is relied upon only as suggesting conjugate formulas comprising two moieties, and does not overcome the failure of Leung and Maloney to suggest a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody. No *prima facie* case of obviousness exists with respect to the combination of Leung, Maloney and Pouletty.

E. The rejection of claims 24-27, 36-38, 43, 44, 52, 55-89, 98 and 99 under 35 U.S.C. §103(a) based on United States Patent No. 5,789,554 in view of Maloney *et al.* and United States Patent No. 5,698,178 ("Goldenberg").

Leung describes immunoconjugates of LL2 with cytotoxic agents or labels (see abstract). The Examiner admits that Leung does not teach combinations of LL2 with anti-CD20 antibodies as recited in claim 24 and claims dependent thereon, but urges that it would have been obvious to combine anti-CD22 immunoconjugates and naked anti-CD20 antibodies based on the disclosure in Maloney of treating B-cell lymphoma, NHL, and other leukemias and lymphomas with a chimeric anti-CD20 monoclonal antibody, rituximab. The Examiner argues that a skilled artisan would have expected a mixture of antibodies to the different epitopes "would be more efficacious in therapeutic methods, as well as enhance the treatment modality," citing the last paragraph on page 2465 of

Maloney. U.S. 5,698,178 is relied upon as teaching specific radioisotopes and toxins, and the Examiner urges that it would have been obvious to administer a therapeutic combination of a known anticancer antibody with other anticancer molecules.

The remarks with respect to Leung and Maloney apply equally here and are incorporated by reference. Goldenberg is relied upon only as suggesting immunoconjugates with radioisotopes, and does not overcome the failure of Leung and Maloney to suggest a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody. No *prima facie* case of obviousness exists with respect to the combination of Leung, Maloney and Goldenberg.

F. <u>The rejection of claims 24-27, 38, 43, 44, 52, 55-89, 98 and 99 under 35 U.S.C. §103(a) based on WO 96/04925 in view of Maloney *et al.* and United States Patent No. 5,698,178 ("Goldenberg").</u>

WO 96/04925 is the published PCT counterpart of Leung, discussed above, and thus similarly describes immunoconjugates of LL2 with cytotoxic agents or labels (see abstract). The Examiner admits that WO 96/04925 does not teach combinations of LL2 with anti-CD20 antibodies as recited in claim 24 and claims dependent thereon, but urges that it would have been obvious to combine anti-CD22 immunoconjugates and naked anti-CD20 antibodies based on the disclosure in Maloney of treating B-cell lymphoma, NHL, and other leukemias and lymphomas with a chimeric anti-CD20 monoclonal antibody, rituximab. The Examiner argues that a skilled artisan would have expected a mixture of antibodies to the different epitopes "would be more efficacious in therapeutic methods, as well as enhance the treatment modality," citing the last paragraph on page 2465 of Maloney. U.S. 5,698,178 is relied upon as teaching specific radioisotopes and toxins, and the Examiner urges that it would have been obvious to administer a therapeutic combination of a known anticancer antibody with other anticancer molecules.

The remarks with respect to Leung and Maloney apply equally here to WO 96/04925, the counterpart of Leung, and Maloney, and are incorporated by reference. Goldenberg is relied upon only as suggesting immunoconjugates with radioisotopes, and does not overcome the failure of Leung and Maloney to suggest a combination of an anti-CD22 immunoconjugate and a naked anti-CD20 antibody. No *prima facie* case of obviousness exists with respect to the combination of WO 96/04925, Maloney and Goldenberg.

G. The provisional rejection of claims 24-27, 36-44, 47, 52, 55-59, 98 and 99 under the judicially created doctrine of obviousness-type double patenting over claims 24-44 of SN 10/314,330.

Claims 24-27, 36-44, 47, 52, 55-59, 98 and 99 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claims 24-44 of SN 10/314,330. This rejection is being held in abeyance until such time as allowable subject matter has been indicated in the second of the two cases to be allowed. MPEP 822.01. Thus, there is no need to review this rejection on appeal.

VIII. CONCLUSION

In summary, it would not have been obvious to combine an immunoconjugate of an anti-CD22 antibody and a naked anti-CD20 antibody. There is no suggestion in the record that would have led a skilled artisan to combination antibody therapy. Moreover, the case of *In re Kerkhoven* clearly has been distinguished on its facts. Appellants have made of record articles and Rule 132 declaration by three well-known experts which establish that antibody therapy, and especially combination antibody therapy, was not conventional at the time the present invention was made. Finally, Appellants have made of record evidence, in the form of several articles, that the combination of antibodies produces results that were unexpected. For all of these reasons, the present claims are believed to be allowable over the outstanding rejections, and the Board is requested to reverse the decision of the Examiner and pass the present case to issuance.

Respectfully submitted,

ROSSI, KIMMS & McDOWELL LLP

JULY 14, 2008
DATE

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CLAIMS APPENDIX

Claims 1-23. Canceled.

24. (Previously presented) A method for treating a subject having a B-cell malignancy, comprising administering to the subject a therapeutic composition comprising a pharmaceutically acceptable carrier, and an immunoconjugate, wherein the immunoconjugate comprises

(i) at least one human, humanized or chimeric anti-CD22 antibody, and

(ii) a drug or a radioisotope,

wherein the immunoconjugate is used in combination with a naked anti-CD20 mAb.

25. (Previously presented) The method according to claim 24, wherein the immunoconjugate comprises a chemotherapeutic drug.

26. (Previously presented) The method according to claim *25,* wherein the chemotherapeutic drug is selected from the group consisting of cyclophosphamide, etoposide, vincristine, procarbazine, prednisone, carmustine, doxorubicin, methotrexate, bleomycin, dexamethasone, phenyl butyrate, bryostatin-1 and leucovorin.

27. (Previously presented) The method according to claim *25,* wherein the chemotherapeutic drug is selected from the group consisting of nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, pyrimidine analogs, purine analogs, antibiotics, epipodophyllotoxins, platinum coordination complexes, and hormones.

28-35. (Canceled)

36. (Previously presented) The method according to claim 24, wherein the anti-CD22 antibody is a human antibody.

37. (Previously presented) The method according to claim 24, wherein the anti-CD22 antibody is a humanized antibody.

38. (Previously presented) The method according to claim 24, wherein the anti-CD22 antibody is a chimeric antibody.

39. (Previously presented)The method according to claim 24, wherein the anti-CD22 antibody comprises a multivalent fusion protein that additionally comprises at least one antibody component that binds with CD19, CD20, CD52 or CD74.

40. (Previously presented) The method according to claim 39, wherein the anti-CD22 antibody comprises a trivalent fusion protein.

41. (Previously presented) The method according to claim 39, wherein the anti-CD22 antibody comprises a tetravalent fusion protein.

42. (Previously presented) The method according to claim 39, wherein the anti-CD22 antibody comprises a quintavalent fusion protein.

43. (Previously presented) The method according to claim 24, wherein the immunoconjugate comprises polyethylene glycol to extend the half-life of the antibody, in blood, lymph, or other extracellular fluids.

44. (Previously presented) The method according to claim 24, wherein the anti-CD22 antibody is hLL2.

45. (Canceled)

46. (Canceled)

47. (Previously presented) The method according to claim 24, wherein the therapeutic composition comprises at least two monoclonal antibodies that bind with distinct CD22 epitopes, wherein the CD22 epitopes are selected from the group consisting of epitope A, epitope B, epitope C, epitope D and epitope E.

48-51. (Canceled)

52. (Previously presented) The method according to claim 24, wherein the radioisotope is selected from the group consisting of ^{98}Au, ^{32}P, ^{125}I, ^{90}Y, ^{186}Re, ^{88}Re, ^{67}Cu, ^{211}At, ^{213}Bi, ^{224}Ac and ^{131}I.

53-54. (Canceled)

55. (Previously presented) The method according to claim 24, wherein the anti-CD22 immunoconjugate comprises a ^{90}Y radioisotope.

56. (Previously presented) The method according to claim 55, wherein the ^{90}Y is attached to the anti-CD22 immunoconjugate by means of chelating agent.

57. (Previously presented) The method according to claim 56, wherein the chelating agent is diethylenetriaminepentaacetic acid.

58. (Previously presented) The method according to claim 38, wherein the radioisotope is ^{67}Cu.

59. (Previously presented) The method according to claim 58, wherein the chelating agent is p-brornoacetamido-benzyl-tetraethylaminetetraacetic acid.

60-97. (Canceled)

98. (Previously presented) The method according to claim 24, wherein the anti-CD20 antibody is administered before the anti-CD22 immunoconjugate.

99. (Previously presented) The method according to claim 24, wherein the anti-CD20 antibody is administered concurrently with the anti-CD22 immunoconjugate.

RELATED PROCEEDINGS APPENDIX

There has been no decision rendered by the Board in the proceeding identified pursuant to paragraph (c)(1)(ii) of this section.

EVIDENCE APPENDIX

Three Declarations were submitted pursuant to §1.132, and are appended. The three declarations were timely submitted and entered into the record on March 20, 2007, in response to a non-final Office Action, and are by the following experts:

Dr. Kenneth Foon
Dr. Myron Czuczman
Dr. John Leonard

Copies are appended of the following articles which were timely submitted and entered into the record on July 25, 2006, with an amendment accompanied by an RCE:

Hiddemann, *Eur. J. Cancer*, 31A(13014):2141-5 (1995).
Skarin *et al.*, *CA Cancer J. Clin.*, 47(6):351-72 (1997).
Webster *et al.*, *Oncology*, 12(5):697-714 (1998).
Czuczman *et al.*, *J. Clin. Oncology*, 17:268 (1999).
Fisher, *Semin Oncol.*, 27(6 Suppl 12):2-8 (2000).
Cheson, *CA Cancer J. Clin.*, 54(5):260-72 (2004).
2003 Merck Manual excerpt on NHL.
Multani *et al.*, *Curr. Pharm. Biotechnol.*, 2(4):279-91 (2001).
Maloney, *Semin. Hematol.*, 37(4 Suppl 7):17-26 (2000).

In addition, copies are appended of the following articles which were timely submitted and entered into the record on October 1, 2007:

Leonard *et al.* *J. Clin. Oncol.*, 23(22): 5044-5051 (2005).
Stein *et al.* *Clin. Cancer Res.*, 10:2868-2878 (2004).
Carnahan *et al.* *Clin. Cancer Res.*, 9:3982s-3990s (2003).
Carnahan *et al.*, *Mol. Immunology*, 44:1331-1341 (2007)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GOLDENBERG

Serial No.: 09/965,796

Filed: October 1, 2001

Title: IMMUNOTHERAPY OF B-CELL MALIGNANCIES USING ANTI-CD22 ANTIBODIES

Group Art Unit: 1643

Examiner: Alana M. Harris

Attorney Docket No.: IMMU:007US3

EFS-WEB

DECLARATION UNDER 37 CFR § 1.132

MAIL STOP AMENDMENT
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

I, Myron Czuczman, being duly warned, declare as follows:

1. I am an Associate Professor of Medicine at the Roswell Park Cancer Institute, Buffalo, New York. I have an extensive background in the field of immunotherapy for cancer treatment, as evidenced by my Curriculum Vitae, which is attached. I have been a key investigator on clinical trials relating to immunotherapy of various B-cell malignancies, particularly rituximab. For example, I am currently a principal investigator for a Phase II trial studying the effects of giving rituximab together with liposomal doxorubicin to patients with relapsed or refractory B-cell non-Hodgkin's lymphoma and also for a Phase III Trial of CHOP plus rituximab versus CHOP plus iodine-131-labeled monoclonal anti-B1 antibody (tositumomab) for treatment of newly diagnosed follicular Non-Hodgkin's Lymphomas. I am also prinicipal investigator for a phase II trial studying of the effects of administering rituximab together with galiximab to patients with stage II, stage III, or stage IV non-Hodgkin's lymphoma.

2. I am familiar with the article Maloney *et al., Blood,* 84(8): 2457-2466 (1994). This article relates to results from a Phase I clinical trial to evaluate the safety of anti-CD20 antibody as a single agent therapeutic.

3. Maloney 1994 states, on page 2465, that "extension of these studies to patients with minimal disease, using antibody alone or in combination with conventional therapies, may provide the greatest benefit. The disclosure in Maloney that anti-CD20 may be combined with a "conventional therapy" would not have suggested to me therapy with a combination of an anti-CD20 antibody and another antibody, such as an anti-CD22 antibody. This is because "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies. The current clinical trials which I referenced above in paragraph 1 both relate to combinations of the anti-CD20 antibody rituximab with a conventional drug therapy (liposomal doxorubicin or CHOP) along the lines envisioned by Maloney 1994.

4. "Conventional" means "conforming to established practice or accepted standards; traditional" (The American Heritage® Dictionary of the English Language: Fourth Edition - 2000). An investigational drug in Phase I clinical trials cannot be considered a conventional therapy, *i.e.*, it does not conform to established practice or accepted standards." By definition, investigational drugs have not been "accepted." Companies can provide investigational drugs to doctors if they are part of a drug trial covered by an FDA-approved protocol, and such drugs are by definition not conventional, since they are not available for use by any doctor on any patient.

5. In 1994 (and later) antibody therapies were not "conventional," and therefore Maloney's comment regarding the addition of "conventional therapies" to his anti-CD20 antibody suggests to a skilled clinician a combination of the anti-CD20 single antibody therapy with a chemotherapy. It would not have suggested therapy with a combination of antibodies. "Conventional therapies," circa 1994 and later, were chemotherapies. The first approved antibody for therapy of any malignancy was the anti-CD20 antibody rituximab that is the subject of Maloney, but it was not approved until 1997, and therefore there was no cancer therapy with any antibody that was a conventional therapy in 1994.

6. Treatment with anti-CD22 antibody was not conventional circa 1994. For example, Goldenberg *et al., J. Clin. Oncol.*, 9: 548-564 (1991) relates to results from a pilot Phase I study involving a small number of patients to see the feasibility of giving this radiolabeled antibody, involving targeting tumor and organs, doses delivered to tumor and

normal organs, and any evidence of efficacy in a small number of patients, and does not establish that treatment with anti-CD22 antibody was "conventional." Juweid *et al. Cancer Res.*, 55:5899s-5907s (1995) is a follow-on report of further treatment of patients with low doses and initial results of high dose therapy in a Phase I trial. Here again, these were early investigational studies and do not represent conventional therapy.

7. Current reviews and texts support the fact that antibody therapy using a combination of antibodies to different targets is not conventional. No such combination therapy has been approved, and even combinations of rituximab with conventional chemotherapy for lymphoma have only been approved by the FDA within the past two years. Although some articles began to discuss the possibility of combination antibody therapies following publication by Immunomedics of their studies of epratuzumab and rituximab in about 2002/2003, none of these indicate that such therapy is "conventional." For example, "What is New in Lymphoma," published in 2004, cites rituximab as an advancement in the treatment of NHL. Cheson, CA *Cancer J Clin*, Sep-Oct; 54(5):260-72 (2004). Efforts to improve the activity of rituximab are noted, and include increasing the number of weekly infusions, delivering higher doses and increasing dose density. Combinations with CHOP are also mentioned. I published a report in 1999 showing that a combination treatment of anti-CD20 monoclonal antibody and CHOP chemotherapy (a "conventional therapy") showed improved efficacy. Czuczman *et al., Journal of Clinical Oncology*, 17:268, (1999). This is a treatment of anti-CD20 antibody and conventional therapy as mentioned in Maloney, but does not suggest combination antibody therapy. The Cheson article also references both a Phase II study of a combination of rituximab with epratuzumab and a phase I/II study of the combination of galiximab and rituximab, each of which were reported in 2003, demonstrating that combination antibody therapy was still very much investigational at this later date.

****

I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

3/6/07
Date

MD
Myron Czuczman, MD

CURRICULUM VITAE
MYRON S. CZUCZMAN, M.D.

March 20, 2007

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Education:

M.D., Pennsylvania State University College of Medicine, 1985

B.S. Biochemistry and Certificate in Russian and East European Studies, Magna Cum Laude; University of Pittsburgh, 1981

Employment History:

Senior Member, Graduate Faculty of the University of Buffalo. 10/21/04-Present

Member, Tumor Immunology Program (emphasis on hematologic malignancies), 7/02 – Present, Roswell Park Cancer Institute, Buffalo, New York

Associate Professor of Medicine, with full tenure, 4/01-present, Roswell Park Cancer Institute, Buffalo, New York

Assistant Professor of Medicine, 7/92-3/01, Roswell Park Cancer Institute, Buffalo, New York

Covering Physician, 7/90-6/92, Urgent Care Center, Memorial Sloan-Kettering Cancer Center.

Covering Physician, 9/90-6/91, Autologous Bone Marrow Transplant Unit, Memorial Sloan-Kettering Cancer Center.

Research Assistant, Summer of 1982 and 1983, Department of Biochemistry, Pennsylvania State University College of Medicine. Laboratory of Dr. Eugene A. Davidson, Ph.D.

Research Assistant, 1980-1981, Department of Biochemistry, University of Pittsburgh School of Medicine. Laboratory of Dr. Robert H. Glew, Ph.D.

HONORS, AWARDS:

Phi Beta Kappa, 1981

NIH Medical Scientist Training Grant, Summer of 1982 and 1983

Memorial Sloan-Kettering Clinical Scholar's Training Grant, July 1990-1992 ($8,550/year)

Selected to the 1999 Edition of "The Best Doctors in America", Woodward / White, Inc.

Department of Medicine "Best Teacher Award" 1999-2000 and 2003-2004: Selected by Hematology/Oncology Fellows

Elected to America' s Registry of Outstanding Professionals, 2002-2003

Elected to Strathmore' s Who' s Who, 2002-2003

Selected to "America' s Top Physicians, 2003"

Nominated as International Health Professional of the Year for 2004, from the International Biographical Centre, Cambridge, England

Selected to the 2001-2002, 2003-2004, 2004-2005 Editions of "The Best Doctors in America", Castle Connolly Medical, Ltd.

Nominated as Man of the Year, 2004, by the Governing Board of Editors of the American Biographical Institute

Chosen for a "Patient Service Award" by The Leukemia & Lymphoma Society (Western New York & Finger Lakes Chapter), December 2005

Selected to represent Buffalo, NY in the 2006 Edition of The Metropolitan Who' s Who Professional Honor Society.

PROFESSIONAL CERTIFICATION:

Diplomate in Internal Medicine
September, 1988

Board Certified in Medical Oncology (#120978)
November, 1991

New York State Licensed Physician:
169807-1

POSTDOCTORAL TRAINING:

Cornell's North Shore University Hospital/Memorial Sloan-Kettering Cancer Center Program, Internship and Residency, 1985-1988

Memorial Sloan-Kettering Cancer Center, Medical Oncology/Hematology Fellow, 1988 - 1992

Laboratory of Hematopoietic Cancer Immunochemistry, Sloan-Kettering Institute, Senior Research Fellow, 1989 - 1992

ACADEMIC APPOINTMENTS:

10/21/04-Present	Senior Member, Graduate Faculty of the University of Buffalo Roswell Park Cancer Institute State University of New York at Buffalo
4/02 – Present	Member, Tumor Immunology Program Roswell Park Cancer Institute State University of New York at Buffalo
4/01 - Present	Associate Professor of Medicine Division of Medicine Departments of Hematologic Oncology and Bone Marrow Transplantation Roswell Park Cancer Institute State University of New York at Buffalo
5/98 - Present	Head, Lymphoma/Myeloma Service Division of Medicine Departments of Hematologic Oncology and Bone Marrow Transplantation Roswell Park Cancer Institute State University of New York at Buffalo
4/97 - 2/98	Medicine Clinic Physician Coordinator Roswell Park Cancer Institute, Buffalo, NY Jerome W. Yates, M.D., M.P.H., Acting Chair, Division of Medicine
7/95 - Present	Medical Staff Buffalo General Hospital 100 High Street Buffalo, New York 14203
5/94 - Present	Adjunct Faculty Physician Assistant Program D'Youville College One D'Youville Square 320 Porter Avenue Buffalo, NY 14201-1084

7/1/92 – 3/01 Assistant Professor of Medicine
Division of Medicine
Departments of Hematologic Oncology and
Bone Marrow Transplantation
Roswell Park Cancer Institute
State University of New York at Buffalo

PROFESSIONAL MEMBERSHIPS:

Member, American Association of Cancer Research
Member, Cancer and Leukemia Group B
Member, American Society of Hematology
Member, American Society of Clinical Oncology
Member, American Cancer Society
Member, National Comprehensive Cancer Center

NATIONAL SERVICE:

Member, National Comprehensive Cancer Network (NCCN) Lymphoma Guidelines Panel, 6/97 - Present

Member (original), National Comprehensive Cancer Network (NCCN) NHL Outcomes Database Panel, 11/99 – Present

Study co-chair of Cancer and Leukemia Group B Protocol 8364 "Immunological Diagnostic Studies in Adult ALL"

Reviewer, American Society of Hematology, Category: Lymphoma Therapy, Excluding Transplantation. Abstracts for 12/99 Meeting

Chair, Session: "Antibody-based Therapy of B-Cell Lymphomas" American Society of Hematology, 12/99 Meeting

Cadre Member, Lymphoma Committee, Cancer and Leukemia Group B (CALGB) 1/00 - Present

National Chair, Cancer and Leukemia Group B (CALGB) Protocol 59901 "A phase II study of 506U78 in patients with previously systemically untreated cutaneous T-cell lymphoma (CTCL) or with refractory or relapsed non-cutaneous peripheral T-cell lymphoma (PTCL). 1/00 – 2004; Manuscript submitted for publication, 11/05

Study Chair of Cancer and Leukemia Group B Protocol 50102/SWOG S0016 "A Phase III Trial of CHOP vs. CHOP + Rituximab vs. CHOP + Iodine-131 Labeled Monoclonal Anti-B1 Antibody (Tositumomab) for Treatment of Newly Diagnosed Follicular Non-Hodgkin' s Lymphoma" 12/15/01 - Present

Organizer and Annual Co-Chairman of the International Workshop on NHL (IWNHL), 1st held in November, 2002 (France); continues as an annual international event

Chair, Harvard Cancer Center' s Drug and Safety Monitoring Committee for Waldenstrom' s multicenter clinical trials. 1/01/02 – 12/31/03

Member, Editorial Board of the American Journal of Oncology Review (AJOR). 01/01/02 – Present

Member, Editorial Board for "NHL Updates". 07/01/02 – Present

Member, Editorial Board for "Targeted Therapies" (www.tgt-therapies.com). 01/01/04 – present.

Member, Editorial Board for "Current Hematologic Malignancy Reports. 11/05 – present

Moderator, ASH 2005: Lymphoma: Therapy with Biologic Agents, excluding pre-Clinical Models 1. (Co-Moderator: Bruce Peterson, MD, U of MN) December 12, 2005. Atlanta, GA.

Ad Hoc Member, NCI PO1 Review Study Group, Hematologic Malignancies Cluster Review, Subcommittee D, 2/06 - present

INSTITUTE / COMMUNITY SERVICE:

Member of Institutional Review Board, RPCI, 8/92 to 9/99; reappointed 10/05 - present

Elected Member of RPCI Medical Staff Executive Committee, 4/95 Representative At Large - 2 years; Re-elected for additional 2 year term, 4/97

Member, Board of Directors, Western District American Cancer Society, New York State Division, 8/95 - 8/99

Member, Operating Room Committee, 6/96 - 7/98

Member, Task Force on Managed Care and Marketing at RPCI since 3/97

Member, Focus Group for Redeveloping the Referral Office at RPCI since 9/97

Member, Critical Care Committee since 6/96 - 8/04

Member, Search Committee for Chairman of Immunology Department, 1998

Elected to Board of Directors, Roswell Park Community Cancer Network IPA, Inc., 2/99 to present

Member, Primary Care Task Force, since 5/99

Elected Member of RPCI Medical Staff Executive Committee, 5/00
Representative At Large - 2 years; Re-elected for additional 2 year term, 4/02

Member, Strategic Leadership Council, RPCI, 6/00

Member, Fellowship Research Review Committee, RPCI, 7/02 – present

Member, Conflict of Interest Task Force, RPCI, 12/02 – present

Member, Health Information/Medical Records Committee, RPCI, 9/04 - 07/05

Member, Clinical Leadership Committee, RPCI 11/00; reappointed 5/1/05 - present

JOURNAL REFEREE:

American Journal of Hematology

American Journal of Oncology Review

Annals of Hematology

Blood

British Journal of Haematology

Cancer

Cancer Investigation

Clinical Cancer Research

Clinical Lymphoma

Journal of Clinical Oncology

Oncology

EDITORIAL BOARD:

The American Journal of Oncology Review

NHL Updates (Darwin Medical Communications)

Current Hematologic Malignancy Reports

DEPARTMENTAL SERVICE:

Coordinator of Hematology/Oncology Module for Physician Assistant students at D'Youville College, 1994-2003.

TEACHING AND PRECEPTORSHIP:

1992 - Present	Attending on inpatient Hematology Oncology/BMT Service with fellows and residents Roswell Park Cancer Institute
1992 - Present	Attending in Medical Oncology Clinic with fellows, medicine residents, and medical students Roswell Park Cancer Institute, 2 days/week
1994 – Present	Lecturer, first year medical student Cancer Selective State University of New York at Buffalo
1994 – 2003	Lecturer, Clinical Medicine II Physician Assistant Program D' Youville College, Buffalo, NY
1996 – Present	Lecturer, Fellows Oncology Syllabus Roswell Park Cancer Institute
1997 – Present	Lecturer, third year medical student Oncology Selective State University of New York at Buffalo
1997 – Present	Preceptor, Summer Medical Student Program Roswell Park Cancer Institute Varada Nargund, Lake Erie Osteopathic College (1997) William Lee, SUNY Health Science Center (1998) John Hayslip, North East Ohio (1998) Jeff Davis, University of North Dakota (1999) David Cipolla (2001) Gregory Connolly (2001) Scott Reising (2002) Adam Kotowski (2003) Adam Schweickert (2004)
1998	Thesis Advisor, Master' s Degree State University of New York at Buffalo Roswell Park Cancer Institute Mark Chester "Comparative sensitivity of an RT-PCR versus PCR-Based assay for the detection of clonal IgH gene rearrangement"
2000	Preceptor, Senior Hematology / Oncology Research Fellow Project Francisco Hernandez, MD "Elucidation of mechanisms of action and resistance pathways of biologically active monoclonal antibodies" (in collaboration with Betsy Repasky, Ph.D., Dept of Immunology, RPCI)
2003 -	Sponsor and Mentor for Jeyanthi Ramanarayan

	ASCO Young Investigator Award Recipient The Laboratory of Translational Lymphoma Research (Head, Myron S. Czuczman, MD) Title of Research: "Targeting Bcl-2 expression in rituximab-resistant lymphoma cells as a therapeutic strategy to overcome monoclonal antibody acquired resistance" Current Position: Assistant Professor of Hematology and Oncology, VA Medical Center, Albany, NY
2003-2004	PESP NY State Research Award (Funding for Translational Cancer Research Training) Recipient: Nishita Reddy, MD Current Position: Chief Fellow, RPCI Hematology / Oncology Fellowship Program
2004 – Present	Co-Mentor and Advisor (along with Dr. James Clements, Department of Immunology) in the Laboratory of Translational Lymphoma Research (Head, Myron S. Czuczman, MD) Ph.D. Candidate: Scott Olejniczak, MS Title of Ph.D. Thesis: "Role of multi-domain Bcl-2 family proteins in shared resistance to rituximab and chemotherapy"
2005-2007	Re-awarded PESP NY State Research Award (2 year term) Recipient: Raymond Cruz, MD

RESEARCH ACTIVITIES:

Clinical research studying monoclonal antibodies either alone or in combination with other agents (i.e. primarily chemotherapy) in the treatment of lymphoid neoplasms.

Clinical research evaluating the significance of minimal residual disease in lymphoid malignancies.

Principal Investigator on several unlabeled and radiolabeled monoclonal antibody clinical trials for non-Hodgkin's lymphoma: Investigator-initiated, pharmaceutical-sponsored, and CALGB-sponsored trials.

Study of immunophenotype of lymphoid neoplasms by multiparameter flow cytometry.

GRANT SUPPORT, Completed:

Memorial Sloan-Kettering Clinical Scholar's Training Grant, July 1990-1992 ($8,550/year)

ACS Grant # PRTF-135, P.I.: Myron S. Czuczman, July 1, 1990 - June 30, 1992, $47,000

NIH CA59518 "Cancer and Leukemia Group B RPCI / SUNY AB" P.I. Ellis Levine, MD, Co-P.I. Myron S. Czuczman, MD. 4/93 – 3/31/98 $767,333.00

NIH CA59518 "Cancer and Leukemia Group B RPCI / SUNY AB" P.I. Ellis Levine, MD, Co-P.I. Myron S. Czuczman, MD. 4/98 – 3/31/00 $487,712.00

Burroughs Wellcome, Co. P.I. Myron S. Czuczman. A Pilot Study of CAMPATH-1H in Patients with Minimal Residual non-Hodgkin's Lymphoma Detectable by Polymerase Chain Reaction. $56,000.00. (Completed.)

Burroughs Wellcome, Co. P.I. Myron S. Czuczman. A Multi-Center Phase II Study of CAMPATH-1H in Patients with non-Bulky non-Hodgkin's Lymphoma. $11,000.00. (Completed.)

IDEC Pharmaceuticals, Corp., P.I. Myron S. Czuczman. Pilot Phase II Study to evaluate the safety and clinical activity of IDEC-C2B8 in combination with chemotherapy (CHOP) in patients with low grade B-cell lymphoma. Activated 4/1/94. $94,500.00. (Completed.)

IDEC Pharmaceuticals, Corp., P.I. Myron S. Czuczman. Phase II multicenter study to evaluate the safety and efficacy of once weekly times 4 dosing of IDEC-C2B8 in patients with relapsed low grade or follicular B-cell lymphoma. Activated 5/1/96. $44,000.00. (Completed.)

IDEC Pharmaceuticals, Corp., P.I. Myron S. Czuczman. Phase II multicenter study to evaluate the safety and efficacy of once weekly times 8 dosing of IDEC-C2B8 in patients with relapsed low grade or follicular B-cell lymphoma. Activated 5/23/96. $18,000.00. (Completed.)

Genentech, Inc. P.I. Myron S. Czuczman. Phase II pilot study of safety and efficacy of IDEC-C2B8 in combination with CHOP chemotherapy in previously untreated patients with intermediate or high grade non-Hodgkin's lymphoma. Activated 10/23/96. $26,000.00. (Completed.)

Immunomedics, Inc., P.I. Myron S. Czuczman. Phase I/II study of ^{131}I-LL2 murine IgG antibody in patients with non-Hodgkin's lymphoma. $47,386.50. (Completed)

IDEC Pharmaceuticals, Corp., P.I. Myron S. Czuczman. A randomized phase III multi-center, controlled trial to evaluate the efficacy and safety of IDEC-Y2B8 Radioimmunotherapy Compared to Rituxan™ immunotherapy of relapsed or refractory low-grade or follicular B-cell non-Hodgkin's lymphoma. $60,000.00 (Completed, 8/99)

NIH RO-1 CA 67026-04 grant, Co-P.I. Myron S. Czuczman, 8/99 – 7/02: Radioimmunotherapy of non-Hodgkin's lymphoma with LL2 monoclonal antibody. *Early studies of ^{90}Yttrium-labeled humanized LL2 (anti-CD22) monoclonal antibody.* Total: $228,770.00 (3 year award to RPCI).

GRANT SUPPORT, Current:

PO1 CA103985-1. National Health Institute. (PI, Project 3) 09/12/2005 to 09/12/2009
"Targeting therapy of B-cell lymphoma". This is a combined effort between three Cancer Institutes to develop new targeted therapies for B-cell malignancies by improving the anti-tumor activity of Monoclonal antibodies. The Participating Institutions are: The Garden State Cancer Center, Fox Chase Cancer Center and Roswell Park Cancer Institute. 10% effort on project.

PESP-New York State program (PI). 07/01/05-7/01/07
Targeting CD52 in rituximab resistant B-cell lymphomas. The major goal of this program is to study the effects of CD52 upregulation in rituximab resistance. In addition this program is intended to foster the development of the academic career of a physician on training to conduct translation research.

BC020043 Department of Defense Breast Cancer Research Program (BCRP). P.I. Ben Seon, Ph.D., co-P.I. Myron S. Czuczman, MD, 4/1/04 – 3/31/08:
Vascular Targeting Anti-Angiogenic Therapy of Human Breast Cancer. 5% effort on project. Total: $3,251,260.00

PESP-New York State program (PI). 07/01/03-7/01/04
Preclinical in vitro and in vivo characterization of the effects of immunomodulatory drugs (IMiDs) on the anti-tumor activity of biologically active monoclonal antibodies directed against Non-Hodgkin's Lymphoma (NHL). The major goal of this program is to study the effects of CC5013 and CC4047 in the anti-tumor activity of rituximab and other monoclonal antibodies. In addition this program is intended to foster the development of the academic career of a physician on training to conduct translation research.

Kern McNeill International, P.I. Myron S. Czuczman (RPCI). The effects of PROCRIT® (Epoetin alfa) on Hemoglobin, symptoms distress and quality of life during chemotherapy in lymphoma patients with mild to moderate anemia. $68,000.00 to date. Accrual completed in 2004. Manuscript submitted for publication.

Genentech Inc. and Berlex Labs, P.I. Myron S. Czuczman. (Single institution study) Phase II pilot study of the safety and efficacy of Rituxan (Chimeric anti-CD20 antibody) in combination with Fludarabine chemotherapy in patients with low-grade or follicular lymphoma. $100,000.00. Accrual completed 12/00. Manuscript published in J Clin Oncol, 2005.

IDEC Pharmaceuticals, Corp., P.I. Myron S. Czuczman. A Phase II open label multi-center trial to evaluate the efficacy and safety of IDEC-Y2B8 radioimmunotherapy in patients with B-cell non-Hodgkin' s lymphoma who have not responded to prior Rituximab therapy. $26,000.00 to date. Accrual completed. Manuscript published in J Clin Oncol, 2002.

Roswell Park Cancer Institute Alliance Foundation Award: Radioimmunotherapy of B-cell lymphoma with ^{90}Y-hLL2 radiolabeled monoclonal antibody. $5,000.00 (for supplies).

IDEC Pharmaceuticals, Corp, P.I. Myron S. Czuczman. Expanded access study of IDEC-Y2B8 radioimmunotherapy of relapsed or refractory low-grade or follicular B-cell non-Hodgkin' s lymphoma. $50,000.00. Accrual completed.

Fannie Rippel Foundation, Co-P.I.' s Myron S. Czuczman, MD (Medicine) and Dominick Lamonica (Nuclear Medicine): Awarded $200,000.00 grant for purchase of dual-head gamma camera imaging system to be utilized for radioimmunotherapy clinical studies at RPCI. January, 2001.

PUBLICATIONS:

1. Glew RH, Diven WF, Zidian JL, Rankin BB, **Czuczman MS**, Axelrod AE. Metabolism of Lysosomal Enzymes in the Protein-Deficient Weanling Rat. Am J Clin Nutr 35(2):236-249. 1982.

2. Glew RH, **Czuczman MS**, Diven WF, Berens RL, Pope MT, Katsoulis DE. Partial Purification and Characterization of Particulate Acid Phosphatase of Leishmania donovani Promastigotes. Comp Biochem Physiology (B) 72:581-590. 1982.

3. Barsoum AL, C**zuczman MS**, Bhavanandan VP, Davidson EA. Epitopes Immunologically Related to Glycophorin A on Human Malignant and Non-malignant Cells in Culture. Int J Cancer 34:789-795. 1984.

4. **Czuczman MS**, Divgi CR, Straus DJ, Lovett D, Garin-Chesa P, Yeh S, Feirt N, Graham M, Leibel S, Gee TS, Myers J, Pentlow K, Finn R, Oettgen HF, Larson SM, Old LJ, Scheinberg DA. 131Iodine-labeled Monoclonal Antibody Therapy of Acute Myelogenous Leukemia and B cell Lymphoma. Antibody, Immunoconj and Radiopharm 4(4):787-793. 1991.

5. **Czuczman MS** and Scheinberg DA. Monoclonal Antibody Therapy for NHL. Contemporary Oncology 2(7):45-52. 1992.

6. **Czuczman MS**, Garin-Chesa P, Lemoli RM, and Scheinberg DA. IgM Monoclonal Antibody JD118 Recognizes an Inducible Antigen Target for Human Complement Mediated Cytotoxicity Against Neoplastic B Cells. Cancer Immunol and Immunother 36:387-396. 1993.

7. **Czuczman MS**, Class K, Scheinberg DA. Interleukin-4 Priming Enhances a Target for Human Complement-Mediated Cytotoxicity of CLL. Leukemia 7(7):1020-1025. 1993.

8. **Czuczman MS**, Straus DJ, Divgi CR, Garin-Chesa P, Finn R, Myers J, Leibel S, Graham M, Larson S, Oettgen HF, Old LJ, and Scheinberg DA. A Phase I Dose Escalation Trial of 131I-labeled Monoclonal Antibody OKB7 in Patients with non-Hodgkin's Lymphoma. J Clin Oncol 11(10):2021-2029. 1993.

9. Berinstein NL, Grillo-Lopez AJ, White CA, Bence-Bruckler I, Maloney D, **Czuczman M**, Green D, Shen D. Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Annals of Oncology 9:995-1001, 1998.

10. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, **Czuczman MS**, Cohen R, Heyman MR, Bence-Bruckler I, Jain V, Ho AD, Lister J, White CA, Cabanillas F, Wey K, Shen D, Dallaire B. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 16:2825-2833, 1998.

11. Leget GA, **Czuczman MS**. Use of rituximab, the new FDA-approved antibody. Current Opinion in Oncology 10:548-551, 1998.

12. **Czuczman MS**, Grillo-Lopez AJ, White CA, Saleh M, Gordon L, LoBuglio AF, Jonas C, Klippenstein D, Dallaire B, and Varns C. Treatment of Patients with Low-Grade B-cell Lymphoma with the Combination of Chimeric Anti-CD20 Monoclonal Antibody and CHOP Chemotherapy. J Clin Oncol 17:268-276, 1999.

13. **Czuczman MS**. Combined modality therapy using CHOP plus Rituxan in patients with low grade lymphoma. Biological Therapy of Lymphoma. 2(1):2-6, 1999.

14. Alidina A, Lawrence D, Ford LA, Baer MR, Bambach B, Bernstein S, **Czuczman MS**, Slack J, Spangenthal E, Wetzler M, Barcos MP, Proulx G, Anderson B, McCarthy Jr. PL. Thiotepa-associated cardiomyopathy during blood or marrow transplantation association: Association with female gender and cardiac risk factors. Biology of Blood and Marrow Transplantation, 5:322-327, 1999.

15. **Czuczman MS**, Dodge RK, Stewart CC, Frankel SR, Davey FR, Powell BL, Szatrowski TP, Schiffer CA, Larson RA, Bloomfield CA. Value of Immunophenotype in Intensively Treated Adult Acute Lymphoblastic Leukemia: Cancer and Leukemia Group B Study 8364. Blood 93(11):3931-3939, 1999.

16. **Czuczman MS**, Adis New Drug Profile: Rituximab, A Viewpoint. Drugs 58(1):89, 1999.

17. **Czuczman MS**, CHOP plus Rituximab Chemoimmunotherapy of Indolent B-cell Lymphoma. Seminars in Oncology 26(5); suppl 14:88-96, 1999.

18. Piro LD, White CA, Grillo-Lopez AJ, Janakiraman N, Saven A, Beck TM, Varns C, Shuey S, **Czuczman M**, Lynch JW, Kolitz JE, Jain V. Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin' s lymphoma. Annals of Oncology 10:655-661, 1999.

19. Proulx GM, El-Agamawi AY, Lee RJ, Orner JB, **Czuczman M**, McCarthy P, Bernstein Z, Bernstein S. Primary non-Hodgkin' s lymphoma of bone: treatment and outcome. Radiol Oncol 34(1): 27-33, 2000.

20. Davis TA, Grillo-Lopez AJ, White CA, McLaughlin P, **Czuczman MS**, Link BK, Maloney DG, Weaver RL, Rosenberg J, Levy R. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin' s lymphoma: Safety and efficacy of re-treatment. J Clin Oncol 18(17):3135-3143, 2000.

21. Benekli M, Anderson B, Wentling D, Bernstein S, **Czuczman M**, McCarthy P. Severe respiratory depression after dimethylsulphoxide-containing autologous stem cell infusion in a patient with AL amyloidosis. Bone Marrow Transplant, 25(12): 1299-1301, 2000.

22. Vose JM, Link BK, Grossbard ML, **Czuczman M**, Grillo-Lopez A, Gilman P, Lowe A, Kunkel LA, Fisher RI. Phase II study of Rituximab in combination with CHOP chemotherapy in patients with previously untreated aggressive non-Hodgkin's lymphoma. J Clin Oncol. 19(2):389-397, 2001.

23. **Czuczman MS,** Grillo-López AJ, McLaughlin P, White CA, Saleh M, Gordon L, LoBuglio AF, Rosenberg J, Alkuzweny B, Maloney D. Clearing of Cells Bearing the bcl-2 [t(14;18)] Translocation from Blood and Marrow of Patients Treated with Rituximab Alone or in Combination with CHOP Chemotherapy. Annals of Oncology. 12:109-114, 2001.

24. Khorana A, Bunn P, McLaughlin P, Vose J, **Czuczman MS**. A Phase II Multicenter Study of CAMPATH-1H Antibody in Previously Treated Patients with Nonbulky Non-Hodgkin's Lymphoma. Leukemia and Lymphoma. 41(1-2):77-87, 2001.

25. Miklos S, Hahn TE, Ford L, Anderson B, Swinnich D, Baer MR, Bernstein SH, Bernstein ZP, **Czuczman MS**, Slack JL, Wetzler M, Herzig G, Schriber J, McCarthy PL. Retrospective analysis of hepatic veno-occlusive disease after blood or marrow transplantation: Possible beneficial use of low molecular weight heparin. Bone Marrow Transplantation 27:627-633, 2001.

26. **Czuczman MS**. Combination chemotherapy and Rituximab. Anti-Cancer Drugs, 12(2):15-19, 2001.

27. Khushalani NI, Bakri FG, Wentling D, Brown K, Mohr A, Anderson B, Keesler C, Ball D, Bernstein, ZP, Bernstein SH, **Czuczman MS**, Segal BH, McCarthy PL. Respiratory syncytial virus infection in the late bone marrow transplant period: report of three cases and review. Bone Marrow Transplantation 27:1071-1073, 2001.

28. Hahn T, Wolff SN, **Czuczman M**, Fisher RI, Lazarus H, Vose JM, Warren L, Watt R, McCarthy PL. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of diffuse large cell B-cell non-Hodgkin's lymphoma: an evidence-based review. Biol Blood Marrow Transplant 7:308-331; 2001.

29. **Czuczman MS**. Rituximab ups survival in Aggressive and Indolent NHL. Oncology News International. 11(1):1-2, 2002.

30. **Czuczman MS**, Fallon A, Mohr A, Stewart C, Bernstein ZP, McCarthy P, Skipper M, Brown K, Miller K, Wentling D, Klippenstein D, Loud P, Rock MK, Benyunes M, Grillo-Lopez AJ, Bernstein SH. Rituximab in combination with CHOP or Fludarabine in low-grade lymphoma. Seminars in Oncology 29(1) (suppl 2): 36-40, 2002.

31. Gordon LI, Witzig TE, Wiseman GA, Flinn IW, Spies SS, Silverman DH, Emmanuolides C, Cripe L, Saleh M, Czuczman MS, Olejnik T, White CA, Grillo-Lopez AJ. Yttrium 90 ibritumomab Tiuxetan radioimmunotherapy for relapsed or refractory low-grade non-Hodgkin's lymphoma. Seminars in Oncology 29(1) (Suppl 2): 87-92, 2002.

32. **Czuczman MS**. Immunochemotherapy in indolent non-Hodgkin's lymphoma. Seminars in Oncology 29(2) (Suppl 6):11-17, 2002.

33. Witzig TE, Gordon LI, Cabanillas F, **Czuczman MS**, Emmanouilides C, Joyce R, Pohlman BL, Bartlett NL, Wiseman GA, Grillo-Lopez AJ, Multani P, White CA. Randomized controlled trial of Yttrium-90-labeled Ibritumomab Tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol, 20(10):2453-2463, 2002.

34. Witzig TE, Flinn IW, Gordon LI, Emmanouilides C, **Czuczman MS**, Saleh MN, Cripe L, Wiseman G, Olejnik T, Multani PS, White CA. Treatment with Ibritumomab Tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's Lymphoma. J Clin Oncol 20(15):3262-3269, 2002.

35. Chanan-Khan A, **Czuczman MS**. Radioimmunotherapy in non-Hodgkin's lymphoma. Curr Opin Oncol 14:484-489, 2002.

36. Hagenbeek A, **Czuczman MS**, Ghielmini M, Herold M, Kimby E, Solal-Celigny P, Unterhalt M. Rituximab therapy for indolent non-Hodgkin's lymphoma. Anti-Cancer Drugs 13(supp 2):S11-S17, 2002.

37. Imrie KR, Linch DC, **Czuczman MS**. Debate on the conservative and aggressive treatment options for the optimal management of indolent non-Hodgkin's lymphoma. Anti-Cancer Drugs 13(supp 2):S19-S24, 2002.

38. Lamonica D, **Czuczman M**, Nabi H, Klippenstein D, Grossman Z. Radioimmunoscintigraphy (RIS) with Bectumomab (Tc99m labeled IMMU-LL2, Lymphoscan) in the assessment of recurrent non-Hodgkin's lymphoma (NHL). Cancer Biotherapy & Radiopharmaceuticals 17(6):689-697, 2002.

39. Benekli M, Hahn T, Shafi F, Qureshi A, Alam AR, **Czuczman MS**, Bernstein ZP, Chanan-Khan AA, Becker JL, McCarthy PL. Effect of rituximab on peripheral blood stem cell mobilization and engraftment kinetics in non-Hodgkin' s lymphoma patients. Bone Marrow Transplant 32(2):139-143, 2003.

40. Hahn T, Rondeau C, Shaukat A, Jupudy V, Miller A, Alam AR, Baer MR, Bambach B, Bernstein Z, Chanan-Khan A, **Czuczman M**, Slack J, Wetzler M, Mookerjee B, Silva J, McCarthy PL. Acute renal failure requiring dialysis after allogeneic blood and marrow transplantation identifies very poor prognosis patients. *Bone Marrow Transplant* 32: 405-410, 2003.

41. Hernandez-Ilizaliturri FJ, Jupudy V, Ostberg J, Oflazoglu E, Huberman A, Repasky E, and **Czuczman MS**. Neutrophils Contribute to the Biological Anti-tumor Activity of Rituximab in a Non-Hodgkin' s Lymphoma Severe Combined Immunodeficiency (SCID) Mouse Model. Clinical Cancer Research 9(16): 5866-5873, 2003.

42. Sharkey RM, Brenner A, Burton J, Hajjar G, Toder SP, Alavi A, Matthies A, Tsai DE, Schuster SJ, Stadtmauer ED, **Czuczman MS**, Lamonica D, Kraeber-Bodere F, Mahe B, Chatal JF, Rogatko A, Mardirrosian G, Goldenberg DM. Radioimmunotherapy of non-Hodgkin' s lymphoma with 90Y-DOTA Humanized Anti-CD22 IgG (90Y-epratuzumab): Do tumor targeting and dosimetry predict therapeutic response? J Nuc Med 44:2000-2018, 2003.

43. **Czuczman M**, Zinzani PL. The first International Workshop on non-Hodgkin' s Lymphoma: Taking a close look at current issues and future developments. Leukemia & Lymphoma 44(3): S1-S4, 2003.

44. Cheson BD, **Czuczman M**, Engert A, Hagenbeek A, Zinzani PL. Report from the second International Workshop on non-Hodgkin' s Lymphoma. Co-Editor and Contributor. NHL Updates 2(3): 1-24, 2003.

45. Chanan-Khan A, Srinivasan S, **Czuczman MS**. Prevention and management of cardiotoxicity from antineoplastic therapy. J Support Oncol 2(3):251-256, 2004.

46. Chanan-Khan A, Islam T, Alam A, Miller KC, Gibbs J, Barcos M, **Czuczman MS**, Paplham P, Hahn T, McCarthy P. Long-term survival with allogeneic stem cell transplant following salvage

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132. **Czuczman MS**, Olejniczak S, Gowda AC, Starostik P, Hernandez-Ilizaliturri FJ. Acquirement of rituximab resistance in lymphoma cell lines is associated with structural changes in the internal domain of CD20 regulated at the post-transcriptional level. Blood 104(11):2280, 2004.

133. Hernandez-Ilizaliturri FJ, Gowda AC, Brombos D, Spear D, **Czuczman MS**. The effects of alemtuzamab (A) dose-sequence upon rituximab (R)-induced reorganization of lipid raft domains and in vitro/in vivo anti-tumor activity. Blood 104(11):2289, 2004.

134. Olejniczak SH, Hernandez FJ, **Czuczman MS**. Acquirement of rituximab resistance is associated with the development of chemotherapy resistance in B-cell lymphoma cells: Evidence of shared pathways of resistance between chemotherapeutic agents and biological therapies. Blood 104(11):2297, 2004.

135. Chanan-Khan AA, Miller KC, McCarthy P, DiMiceli LA, Yu J, Bernstein ZP, **Czuczman MS**. A Phase II study of velcade (V), Doxil (D) in combination with low-doise thalidomide (T) as salvage therapy for patients (pts) with relapsed (rel) or refractory (ref) multiple myeloma (MM) and Waldenstrom Macroglobulinemia (WM): Encouraging preliminary results. Blood 104(11): 2421, 2004.

136. **Czuczman MS**, Porcu Pierluigi, Johnson J, Niedzwiecki D, Canellos GP, Cheson BD. CALGB 59901: Results of a Phase II study of 506U78 in CTCL and PTCL. Blood 104(11):2486, 2004.

137. **Czuczman M**, Leigh BR, Witzig TE, Vose JM, Younes A, Alkuzweny B, Larocca A, Cao X, Thall A. Fc-Gamma receptor IIIa and interferon-gamma SNPs do not predict responsiveness of follicular lymphoma to galiximab (anti-CD80 antibody). Blood 104(11):3292, 2004.

138. Chanan-Khan AA, Miller KC, McCarthy P, Koryzna A, Kouides P, Donohue K, Mohr A, Bernstein ZP, Alam A, **Czuczman MS**. VAD-t (vincristine, adriamycin, dexamethasone and low-dose thalidomide) is an effective initial therapy with high response rates for patients with treatment naïve multiple myeloma (MM). Blood 104(11):3463, 2004.

139. Chanan-Khan AA, Miller Kena, Koryzna A, Takeshita K, McCarthy P, Mohr A, Donohue K, Bernstein ZP, Alam A, **Czuczman MS**. Thalidomide (T) in combination with fludarabine (F) as initial therapy for patients (pts) with treatment naïve chronic lymphocytic leukemia (CLL): Results of a Phase I trial. Blood 104(11):3476, 2004.

140. Hernandez FJ, Reddy N, Nallapareddy S, **Czuczman MS**. Biological effects of rabbit anti-thymocyte immunoglobulin (rATG, thymoglobulin ®) in T-cell non-Hodgkin' s lymphoma cell lines. Blood 104(11):4628, 2004.

141. Wilson WH, Porcu P, Hurd D, Martin SE, **Czuczman M**, Niedzwiecki D, Saint Louis JD, Johnson JL, Cheson B, Canellos GP, Zelenetz AD. Phase II study of dose-adjusted EPOCH-R in untreated de novo CD20+ diffuse large B-cell lymphoma (DLBCL)-CALGB 50103. Proc ASCO 23:6530, 2005.

142. Miller KC, **Czuczman MS**, Dimiceli L, McCarthy P, Bernstein ZP, Zeldis JB, Mohr A, Chanan-Khan AA. Antileukemic effects of novel immunomodulatory agent lenalinomide (L) with or without rituximab (R) in patients (pts) with relapsed (rel) or refractory (ref) chronic lymphocytic leukemia (CLL). Encouraging preliminary results of an ongoing phase II clinical study. Proc. ASCO 23:6557, 2005.

143. Chanan-Khan A, Miller KC, DiMiceli L, Padmanabhan S, Lawrence, Bernstein ZP, Takeshita K, Spaner D, Byrne C, Chrystal C, and **Czuczman MS**. Results of phase II study of Lenalidomide (L) {Revlimid®} in patients with relapsed or refractory chronic lymphocytic leukemia (CLL). Blood 106(11):447, 2005.

144. Younes A, Vose JM, Zelenetz AD, Smith MR, Burris H, Ansell S, Klein J, Kumm E, **Czuczman M**. Results of a Phase 2 trial of HGS-ETR1 (Agonistic Human Monoclonal Antibody to TRAIL Receptor 1) in subjects with relapsed/refractory non-Hodgkin' s lymphoma (NHL). Blood 106(11):489, 2005.

145. Rodriguez MA, **Czuczman MS**, Friedberg JW, Koh ME, LaCasce AS, Millenson M, Auayport N, Niland J, Veer AT, Zelenetz AD, Weeks JC. Age significantly influences presentation and patterns of care in patients with newly diagnosed diffuse large cell lymphoma (DLCL): Collective data from 5 centers participating in the National Comprehensive Center Network (NCCN) NHL Outcomes Project. Blood 106(11):1335, 2005.

146. Hernandez-Ilizaliturri FJ, Devineni S, Arora S, Knight J, **Czuczman MS**. Targeting CD20 and Cd22 with rituximab in combination with CMC-544 results in improved anti-tumor activity against non-Hodgkin' s lymphoma (NHL) pre-clinical models. Blood 106(11):1473, 2005.

147. Hernandez-Ilizaliturri FJ, Olejniczak SH, Knight J, **Czuczman MS**. Structural changes in the internal domain of CD20 antigen associated with the emergence of rituximab resistance: Effects of proteasome inhibition in CD20 structure and rituximab anti-tumor activity in rituximab-resistant cell lines (RRCL). Blood 106(11):1474, 2005.

148. **Czuczman MS**, Maddipatla S, Knight J, Hernandez-Ilizaliturri FJ. In vitro synergistic anti-tumor activity by targeting death-receptor 4 (DR-4) and Cd20 antigen by combining HGS-ETR1 and rituximab monoclonal antibodies (mAbs) against non-Hodgkin' s lymphoma cells (NHL). Blood 106(11):1475, 2005.

149. Friedberg JW, Leonard JP, Younes A, Fisher DC, Gordon LI, Moore JO, **Czuczman** MS, Miller TP, Stiff PJ, Cheson BD, Forero-Torres A, Finucane DM, Leigh BR, Molina A. Updated results from a phase II study of Galiximab (anti-CD80) in combination with rituximab for relapsed or refractory, follicular NHL. Blood 106(11):2435, 2005.

150. LaCasce A, Niland J, Kho ME, terVeer A, Friedberg JW, Rodriguez MA, **Czuczman MS**, Millenson M, Zelenetz AD, Nademanee AP, Weeks JC. Potential impact of pathologic review on therapy in non-Hodgkin' s lymphoma (NHL): Analysis from the National Comprehensive Cancer Network (NCCN) NHL Outcomes Project. Blood 106(11):2816, 2005.

151. Chanan-Khan A, Miller K, Marshall P, Padmanabhan S, Brady W, Bernstein ZP, Wallace P, **Czuczman MS**. Thalidomide (T) in combination with Fludarabine (F) as initial therapy for patients (pts) with treatment naïve chronic lymphocytic leukemia (CLL): Preliminary results of a phase I/II clinical trial. Blood 106(11):2974, 2005.

152. Olejniczak SH, Hernandez-Ilizaliturri FJ, Clements JL, **Czuczman** MS. Lack of lipid raft domain reorganization following ligation of CD20 is associated with resistance to rituximab-induced complement-dependent cytotoxicity. Blood 106(11):4818, 2005.

153. Olejniczak SH, Hernandez-Ilizaliturri FJ, Clements JL, **Czuczman** MS. Loss of expression of the pro-apoptotic Bcl-2 family proteins Bak and Bax in rituximab- and chemotherapy-resistant non-Hodgkin' s lymphoma cells. Blood 106(11):4819, 2005.

154. Hernandez-Ilizaliturri FJ, Kaur H, Bhinder A, Olejniczak S, Knight J, **Czuczman MS**. Impaired Ca++ mobilization in rituximab-resistant cells (RRCL) is associated with changes in the structure of CD20 antigen, down-regulation of Bax/Bak pro-apoptotic proteins and up-regulation of the endoplasmic reticulum (ER) Ca++ pump protein SERCA-3. Proc ASCO 24:2516, 2006.

155. Miller K, **Czuczman MS**, Dimiceli L, Padmanabhan S, Lawrence D, Bernstein Z, Takeshita K, Spaner D, Byrne C, Crystal C, Chanan-Khan AA. Lenalidomide (L) induces high response rates with molecular remission in patients (pts) with relapsed (rel) or refractory (ref) chronic lymphocytic leukemia (CLL). Proc ASCO 24:6517, 2006

156. Reddy NM, Hernandez-Ilizaliturri FJ, Olejniczak S, Kinght J, **Czuczman MS**. Rituximab resistance and its associateion with changes in the internal domain of CD20 antigen and down-regulation of pro-apoptotic protein Bax and Bak in borth rituximab-resistant cell lines (RRCL) and diffuse large B-cell lymphoma (DLBCL) patient (pt) samples. Proc ASCO 24:17509, 2006.

156. Block AW, Wallace PK, Miller KC, **Czuczman MS**, Chanan-Khan AA, Padmanabhan S. Correlation among poor prognostic indicators in B-cell chronic lymphocytic leukemia (B-CLL). Am Soc of Human Genentics. New Orleans, LA October 9-13, 2006.

157. Chanan-Khan AA, Miller KC, Musial L, Padmanabhan S, Lawrence D, Bernstein ZP, Takeshita K, Spaner D, Byrne C, Chrystal C, **Czuczman MS**. Clinical efficacy of lenalidomide (Revlimid) in patients with relapsed or refractory chronic lymphocytic leukemia (CLL): Updated results of a phase II clinical trial. Blood 108(11):306, 2006.

158. Chanan-Khan AA, Padmanabhan S, Miller KC, Musial L, Yu J, Bernstein ZP, Manochakian R, **Czuczman MS**. Final results of a phase II study of bortezomib (velcade) in combination with liposomal doxorubicin (Doxil) and thalidomide (VDT) demonstrate a sustained high response rates in patients (pts) with relapsed (rel) or refractory (ref) multiple myeloma. Blood 108(11):3539, 2006.

INVITED LECTURES / COURSES TAUGHT:

Local / Regional (last 6 years):

"Biotherapy of Cancer." SUNY at Buffalo School of Nursing graduate students. RPCI, Buffalo, NY January 31, 1994.

"Introduction to Hematology." D'Youville College, Physician Assistant Program, Buffalo, NY. April 18, 1994.

"Monoclonal Antibodies and Their Use in Clinical Medicine." SUNY at Buffalo/RPCI Nursing Continuing Education Program. RPCI, Buffalo, NY. October 14, 1994.

"Introduction to Hematology/Physiology of the Blood." D'Youville College, Physician Assistant Program, Buffalo, NY. April 6, 1995.

"Lymphoma" Millard Fillmore medical residents. Buffalo, NY. February 20, 1996.

"Current Treatment of GI Lymphomas" GI Grand Rounds, Buffalo General Hospital. Buffalo, NY. April 16, 1996.

"New Strategies in the Treatment of Low Grade Non-Hodgkin's Lymphoma." BGH Hematology/Oncology Grand Rounds. Buffalo, NY. November 12, 1996.

"Monoclonal Antibody Therapy of NHL". Oncology Grand Rounds. Strong Memorial Hospital. Rochester, NY. January 22, 1998.

"Monoclonal Antibody Therapy of NHL". Oncology Grand Rounds. SUNY at Syracuse. Syracuse, NY. January 22, 1998.

"Chimeric anti-CD20 monoclonal antibody therapy of low grade B-cell lymphoma". Regional Cancer Center Consortium for Biological Therapy of Cancer, Roswell Park Cancer Institute. Buffalo, NY. February 20, 1998.

An update on the use of monoclonal antibodies for the treatment of low grade non-Hodgkin's lymphoma. Community Physician Program. Buffalo, NY. February 24, 1998.

"Immunotherapy of non-Hodgkin's lymphoma: Recent advances". Summer Cancer Lecture Series, 1998. Chautauqua, NY. August 13, 1998.

"Immunotherapy of non-Hodgkin's lymphoma: Recent advances". Grand Rounds, Sisters of Charity Hospital, Buffalo, NY. September 8, 1998.

"Immunotherapy of non-Hodgkin's lymphoma: recent advances". The New York Cancer Society Symposium on Monoclonal Antibody Therapy for Lymphoma. New York, NY February 3, 1999.

"Update on Treatment of Relapsed NHL." Hematology/Oncology Grand Rounds. Memorial Sloan Kettering Cancer Center. New York, NY. June 22, 1999.

"Can Indolent B-cell Lymphoma be Cured? Integrating Monoclonal Antibodies into Current Treatment Strategies" Roswell Park Cancer Institute Medical Grand Rounds. Buffalo, NY February 25, 2000.

"Combination Chemotherapy and Rituximab." Monoclonal Antibodies in the Treatment of Lymphoma and Beyond. Satellite Symposium. New York, NY July 15, 2000.

"Monoclonal antibodies in B-cell lymphoma: Clinical applications." Pharmacy Oncology Symposium 2000. Buffalo, NY. October 27, 2000.

"Monoclonal Antibody Therapy of B-Cell Lymphoma: Review and Update". The Central New York Academy of Medicine's Dr. Lemuel Bowden Cancer Teaching Day. Utica, NY. May 17, 2001.

"Novel Strategies for the treatment of B cell lymphoma: Current status". Medicine Grand Rounds, SUNY Health Science Center. Syracuse, NY. August 15, 2002.

“Lymphoma Case Presentations”. Hem/Onc Conference. SUNY Health Science Center. Syracuse, NY. August 15, 2002.

“NHL - Update and ASH 2002 Lymphoma Review”. Century Medical Associates Oncology Tumor Board Meeting. Buffalo, NY. March 3, 2003.

“A Focus on Radioimmunotherapy – New Therapeutic Options in the Management of Non-Hodgkin’ s Lymphoma”. Institute for Continuing Healthcare Education. Buffalo, NY. April 8, 2003.

“Follicular Lymphoma”. NCCN Non-Hodgkin’ s Lymphoma Regional Guidelines Symposium. Buffalo, NY November 13, 2003.

“Indolent Lymphoma: Review and Update”. Advances in Hematologic Malignancies: A Comprehensive Review. Buffalo, NY. March 6, 2004.

“Meet the Expert on NHL”. Leukemia & Lymphoma Society. Buffalo, NY. October 7, 2004

“Major Advances in the Treatment of B-cell Lymphoma”. City Wide Grand Rounds, Buffalo, NY January 20, 2005.

“Antibody Therapy for Lymphoma”. Roswell Park Cancer Institute 2005 Trainee Lecture Series. Buffalo, NY April 5, 2005.

“Meet the Expert on NHL”. Leukemia & Lymphoma Society. Buffalo, NY. October 17, 2005.

National (last 6 years):

"ImmuRAIT -LL2 Lymphoma Therapy" (oral presentation). Society of Nuclear Medicine. New Vistas in Monoclonal Antibody Imaging and Therapy: Cancer and Infectious Diseases. Orlando, FL. June 4, 1994.

"Radioimmunotherapy of B-Cell Lymphomas with ^{131}I-Labeled LL2 Monoclonal Antibody" (oral presentation). Fifth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer. Princeton, NJ. October 8, 1994.

"Chemoimmunotherapy of low-grade lymphoma with the anti-CD20 antibody IDEC-C2B8 in combination with CHOP chemotherapy" (oral presentation). Chemotherapy Foundation Symposium XIII: Innovative Cancer Therapy for Tomorrow. New York, NY. November 3, 1995.

*"IDEC-C2B8/CHOP Chemoimmunotherapy in patients with low-grade lymphoma: clinical and bcl-2 (PCR) final results" (oral presentation). American Society of Hematology. Orlando, FL. December 9, 1996.

"Recent Advances in Monoclonal Antibody Therapy of Lymphoma" (oral presentation). Fourth International Congress on Biological Response Modifiers. San Antonio, TX. March 13, 1997.

"Will Unconjugated Monoclonal Antibody (mAb) Products be Approved as Cancer Treatment?" (oral presentation). Fourth International Congress on Biological Response Modifiers. San Antonio, TX. March 13, 1997.

"Is anti-CD20 plus chemotherapy more effective than chemotherapy in indolent lymphomas?" Lymphoma. . . The Next Questions. (oral presentation) New Orleans, LA. October 3, 1997.

"IDEC-C2B8 alone and in combination with CHOP in low grade B-cell lymphoma." Chemotherapy Foundation Symposium XV. (oral presentation) New York City, NY. November 12, 1997.

"Immunotherapy of low grade B-cell lymphoma with IDEC-C2B8". Grand Rounds, University of Pittsburgh. Pittsburgh, PA. February 5, 1998.

"Update on monoclonal antibody therapy of NHL". Grand Rounds, University of Arkansas, Little Rock, AR. February 12, 1998.

"Antibody Therapy of non-Hodgkin's Lymphoma." 16th Annual Conference: Clinical Hematology and Oncology. (oral presentation) San Diego, CA. February 18, 1998.

Use of Monoclonal Antibodies in the Treatment of Lymphoid Malignancies." Lymphoid Malignancies in the New Millennium Symposium. (oral presentation) Morristown, NJ. September 9, 1998.

"Monoclonal antibodies for Lymphoma." Grand Rounds. The Rush Cancer Institute. Chicago, IL. October 30, 1998.

"Monoclonal antibodies for Lymphoma." Update in Leukemia and Lymphoma Annual Conference. Sponsored by the Cleveland Clinic Foundation. Cleveland, OH November 6, 1998.

"Rituxan monoclonal antibody therapy of B-cell lymphoma." Chemotherapy Foundation Symposium XVI. (oral presentation) New York City, NY. November 11, 1998.

"Monoclonal Antibodies: New therapeutic approaches in non-Hodgkin's lymphoma." Allogeneic Stem Cell Transplantation Conference. University of Nebraska Medical Center. Omaha, Nebraska. April 30, 1999.

"Rituxan in Combination Therapy of Low Grade B Cell Lymphoma". Chemotherapy Foundation Symposium XVII: Innovative Cancer Therapy for Tomorrow. New York, NY. November 3, 1999.

*"Rituximab/CHOP chemoimmunotherapy in patients (pts) with low grade lymphoma (LG/F NHL): Progression free survival (PFS) after three years (median) follow-up." Selected for Poster Discussion Session, 1999 American Society of Hematology Meeting, New Orleans, LA, December, 1999.

"Monoclonal Antibody Therapy in Non-Hodgkin's Lymphoma." Medical Grand Rounds, University of Maryland Greenbaum Cancer Center, Baltimore, MD February 7, 2000.

"Combination of Rituxan and CHOP in Patients with Low Grade Lymphoma (LG/F NHL): Progression-free Survival after Three Years (median) Follow-up. International Conference on Advances in Cancer Immunotherapy. Princeton, NJ March 2, 2000.

"New modalities of therapy for non-Hodgkin' s lymphoma". Grand Rounds, Mercy Hospital. Pittsburgh, PA. April 13, 2000.

"Advances in monoclonal antibody therapy for non-Hodgkin' s lymphoma". Hematology Grand Rounds. University of Southern California. Los Angeles, CA. May 5, 2000.

"Integrating mAbs into therapy for non-Hodgkin' s lymphoma". Integrating monoclonal antibody therapy into cancer therapy strategies: Recent insights and prospects for Oncology in the 21st Century. American Society of Clinical Oncology 36th Annual Meeting. New Orleans, LA. May 19, 2000.

"Rituximab Immunotherapy: Review and Update" Chemotherapy Foundation Symposium XVIII, Innovative Cancer Therapy for Tomorrow. New York City, NY November 8, 2000.

"Rituximab in Combination with CHOP or Fludarabine in Low-Grade Lymphoma" Recent Advances and Future Directions with Rituxan (Rituximab). Kona, HI. January 12, 2001.

"Diffuse Large B-Cell Lymphoma – Choice of Therapy" Challenging Cases in Hematology by Network for Oncology Communication and Research. Atlanta, GA., April 20, 2001.

"Follicular Lymphoma – 1st Relapse" Challenging Cases in Hematology by Network for Oncology Communication and Research. Atlanta, GA., April 20, 2001.

"Update and Review of Monoclonal Antibodies in B Cell Malignancies". The University of Chicago, Visiting Professor in Oncology. Chicago, IL., June 18, 2001.

"Innovative Therapy of Non-Hodgkin's Lymphoma." Cancer Center Grand Rounds. Karmanos Cancer Institute. Detroit, MI. September 27, 2001.

"Update and Review of Monoclonal Antibodies in B-Cell Malignancies." 7th Annual Jaffar Oncology Conference. Strategies for a Cure: Innovations in Cancer Treatment for the 21st Century. Dearborn, MI. September 28, 2001.

"Radioimmunotherapy for Lymphoma." 2002 Joint Cancer Conference of the Florida Universities. Orlando, FL. February 1, 2002.

"Lymphoma – Low Grade", ASH Highlights #3. New Orleans, LA. March 2, 2002.

"Indolent Lymphoma – Front-Line and First-Relapse". The Visiting Faculty Program on B-Cell Hematologic Malignancies and Disorders: Case Study Development/Review Meeting. American Society of Clinical Oncology. Orlando, FL. May 16, 2002.

"New Strategies for the Treatment of Low-Grade Lymphoma: Current Status". Indolent Lymphoid Malignancies: New Challenges and Therapeutic Advances. American Society of Clinical Oncology. Orlando, FL. May 17, 2002.

"Monoclonal Antibodies in Combination with Chemotherapy for the Treatment of Non-Hodgkin's Lymphoma". Institute for Continuing Healthcare Education. New York, NY. June 11, 2002.

"Monoclonal Antibody Therapy: Review and Update". Grand Rounds, St. Luke's-Roosevelt Hospital Center. New York, NY. June 12, 2002.

"Expanding options in the treatment of non-Hodgkin's lymphoma". The Regional Cancer Center. Erie, PA. September 26, 2002.

"Lymphoma: State of the art". International Oncology Network Meeting. Newark, NJ. October 10, 2002.

"New initiatives in low grade lymphoma". CALGB Fall Group Meeting. Tampa, FL. November 8, 2002.

"ASH 2002 Updates: Low-grade non-Hodgkin's lymphoma." Third Annual Rush Review. Chicago, IL. February 22, 2003.

"Indolent Non-Hodgkin's Lymphoma". A Comprehensive Board Review in Hematology and Medical Oncology. Dallas, TX October 9, 2003.

"Diffuse Large B-cell Lymphoma – Initial Therapy". Network for Oncology Communication and Research Roadmap to Follicular Lymphoma – Challenging Cases in Hematology. New York, NY. November 15, 2003.

"Indolent Lymphoma". International Oncology Network ASH Review. Orlando, FL. January 23, 2004.

"Update: Non-Hodgkin's Lymphoma". National Comprehensive Cancer Network's 9th Annual Conference: Clinical Practice Guidelines and Outcomes Data in Oncology. Hollywood, FL. March 13, 2004.

"Rituximab Translational Research Studies: Making a Good Thing Better!" Joint Lymphoma and Bone Marrow Transplantation Translational Research Seminar. Dallas, TX. May 26, 2004.

"Diffuse Large B-cell Lymphoma". Network for Oncology Communication and Research: Challenging Cases in Hematology. Chicago, IL September 18, 2004.

"New Therapeutic Options in the Management of Non-Hodgkin's Lymphoma" Advances in the Treatment of Non-Hodgkin's Lymphoma: A Focus on Radioimmunotherapy. Ford City, PA. September 20, 2004.

"Indolent Non-Hodgkin's Lymphomas". Comprehensive Board Review in Hematology and Medical Oncology. MD Anderson Cancer Center. October 2, 2004.

"Treatment Options for Patients with Low Grade Non-Hodgkin's Lymphoma". 2nd Annual Symposium on Hematologic Malignancies. Charleston, SC. October 3, 2004.

"Lymphoma Overview". Christiana Care Health Services, Helen F. Graham Cancer Center, Delaware Community Clinical Oncology Program Summit. Wilmington, DE. October 14, 2004.

"Mantle Cell Lymphoma". Network for Oncology Communication and Research, Challenging Cases in Hematology. New York, NY. October 16, 2004.

"Indolent Lymphoma and RIT". International Oncology Network, ASH Review. Orlando, FL. January 15, 2005.

"Low-grade Non-Hodgkin's Lymphoma". 5th Annual RUSH Review. Chicago, IL. January 29, 2005.

"Sunday Brunch with the Experts: Non-Hodgkin's Lymphoma". National Comprehensive Cancer Network's 10th Annual Conference. Hollywood, FL. March 20, 2005.

"Innovations in the Treatment of Indolent and Aggressive Lymphomas". The 1112th Cancer Seminar of The Southern California Academy of Clinical Oncology (SCACO). Century City, CA. June 8, 2005.

"Follicular Lymphoma: Advanced Disease". 2005 Pan Pacific Lymphoma Conference. Kauai, Hawaii. July 13, 2005.

"Transformed Lymphoma". 2005 Pan Pacific Lymphoma Conference. Kauai, Hawaii. July 13, 2005.

"Update and review of CALGB 50402: Extended induction therapy of galiximab plus rituximab in previous untreated follicular lymphoma patients." GALGB Fall Meeting. Amelia Island Plantation, FL. November 18, 2005.

International (last 6 years):

"IDEC-C2B8/CHOP chemoimmunotherapy in patients with low grade lymphoma: Interim clinical and bcl-2 (pcr) results." Poster presentation at the 9th NCI-EORTC Symposium on New Drugs in Cancer Therapy. Amsterdam, Netherlands. March 13, 1996.

"The anti-CD20 antibody IDEC-C2B8 clears lymphoma cells bearing the t(14;18) translocation (bcl-2) from the peripheral blood and bone marrow of a proportion of patients with low-grade or follicular non-Hodgkin's lymphoma" (poster presentation at European Society of Medical Oncology annual meeting). Vienna, Austria. November 3, 1996.

"U.S. Experience with IDEC-C2B8 monoclonal antibody and future trials." European Lymphoma Advisory Panel. Lisbon, Portugal. January 11-15, 1997.

"Update on CALGB 8364: Immunophenotyping of Adult ALL". CALGB Spring Meeting. Montreal, Quebec. June 20-22, 1997.

"IDEC-C2B8/CHOP chemoimmunotherapy in patients with low grade lymphoma: Clinical and bcl-2 (PCR) results." Satellite symposium to XIX symposium of the International Association for Comparative Research on Leukemia and Related Disorders. Mannheim, Germany. July 13, 1997.

"New approaches in the treatment of lymphoma: U.S. experience with IDEC-C2B8 (Rituximab) in the treatment of indolent lymphoma." Brazilian Congress. Sao Paulo, Brazil. August 14-18, 1997.

"U.S. experience with the CD20 antibody, Rituximab, as single agent treatment in indolent lymphoma." International Society of Experimental Hematology Meeting. Cannes, France. August 24-29, 1997.

"Rituximab as combination therapy - the US experience." Innovative antibody therapies for lymphoma. Hamburg, Germany. September 14, 1997.

"Clinical results of anti-CD20 MAB in combination with CHOP in relapsed B-cell Lymphoma." Annual Joint Meeting of the German and Austrian Societies for Hematology and Oncology. Linz, Austria. October 14, 1997.

"Modern Therapies for Low Grade Lymphoma". National Meeting of Heads of Hematology / Oncology Centers in Poland. Warsaw, Poland. March 13, 1998.

"The Role of MabThera in Indolent NHL". IX Congress of the International Society of Hematology, Asian-Pacific Division. Bangkok, Thailand. October 24-28, 1999.

"Combination chemotherapy and MabThera". European Hematology Association-5 Congress, Birmingham, England. June 25-28, 2000.

"Treatment of low-grade and aggressive B-cell lymphoma with the combination of chemotherapy plus Rituximab". German and Austrian Society of Hematology and Oncology (DOGHO). Graz, Austria. October 22-24, 2000.

"Overview of chemoimmunotherapy of NHL" German and Austrian Society of Hematology and Oncology (DOGHO). Graz, Austria. October 22-24, 2000.

"Immunochemotherapy of Indolent NHL" European Hematology Association-6 Congress. Frankfurt, Germany. June 21, 2001.

"Zevalin™ Radioimmunotherapy (RIT) of Rituxan-Refractory Follicular non-Hodgkin's Lymphoma" European Hematology Association-6 Congress. Frankfurt, Germany. June 23, 2001.

"Indolent Lymphoma Debate: Conservative or Aggressive Management?" MabThera Present and Future Time to Re-assess. Montreux, Switzerland. October 6, 2001.

"Updated Results from the Pivotal Study." MabThera Present and Future Time to Re-assess. Montreux, Switzerland. October 6, 2001.

"Rituximab (MabThera™) Combination Immunochemotherapy in B-Cell Lymphoma" 43rd Annual Meeting of the Japanese Society of Clinical Oncology. Kobe, Japan. November 13, 2001.

"Rituximab (MabThera™) Monotherapy of B-Cell Lymphoma" 43rd Annual Meeting of the Japanese Society of Clinical Oncology. Kobe, Japan. November 13, 2001.

"Treatment of Low and Intermediate/High Grade B-cell Lymphoma with the Combination of Chemotherapy plus Rituximab." New Therapy Strategies of Non-Hodgkin's Lymphoma, German Cancer Congress. Berlin, Germany. March 11, 2002.

"Results of Retreatment with MabThera for Patients with Non-Hodgkin's Lymphoma." New Therapy Strategies of Non-Hodgkin's Lymphoma, German Cancer Congress. Berlin, Germany. March 11, 2002.

"Update on the use of MabThera in mantle cell lymphoma." MabThera Scientific Meeting, MabThera: Realising Therapeutic Potential. Noosa, Australia. February 7, 2003.

"Use of MabThera in combination immunotherapy for indolent lymphomas." MabThera Scientific Meeting, MabThera: Realising Therapeutic Potential. Noosa, Australia. February 8, 2003.

"Rituximab in Low-Grade and Mantle-Cell NHL: Review and Update". Annual Meeting of the Quebec Association of Hematologists and Oncologists. Quebec, Canada. March 29, 2003.

"Treatment of the Indolent NHL Patient". 2003 Eastern Canadian Oncology Summitt. Muskoka, Ontario. September 7, 2003.

"MabThera plus CHOP or Fludarabine: An Update on Two Phase II Trials". Evolution and Revolution in NHL Therapy. Monte Carlo. November 1, 2003.

"Indolent Lymphoma: Past, Present and Future". Grand Rounds, Kitchener Cancer Center. Kitchener, Ontario. May 13, 2004.

"Indolent B-cell Lymphoma: Past, Present, and Future". Lymphoma Tumor Group, Vancouver Centre of the British Columbia Cancer Agency. June 25, 2004.

"Case Studies in the Treatment of Indolent B-cell Lymphoma: What would you do?". Lymphoma Tumor Group, Vancouver Centre of the British Columbia Cancer Agency. June 25, 2004.

"Human Polyclonal Antibodies" 3rd International Workshop on Non-Hodgkin's Lymphoma. Stresa, Italy. August 29, 2004.

"Rituximab-Containing Immunochemotherapy of B-Cell Lymphoma" The 67th Annual Meeting of the Japanese Society of Hematology (JSH) and the 4th Annual Meeting of the Japanese Society of Clinical Hematology (JSCH). Tokoyo, Japan. September 17, 2005.

"Monoclonal Antibodies - CD80, Galiximab" Meeting New Drugs in Hematology, Bologna, Italy. October 10, 2005.

"New developments in the treatment of lymphoma." Ontario Canada Cancer Centers (London, Windsor, Cambridge, and St. Catherines). November, 2005.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GOLDENBERG

Serial No.: 09/965,796

Filed: October 1, 2001

Title: IMMUNOTHERAPY OF B-CELL MALIGNANCIES USING ANTI-CD22 ANTIBODIES

Group Art Unit: 1643

Examiner: Alana M. Harris

Attorney Docket No.: IMMU:007US3

EFS-WEB

DECLARATION UNDER 37 CFR § 1.132

MAIL STOP AMENDMENT
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

I, Kenneth Foon, being duly warned, declare as follows:

1. I am the Director of Clinical Investigation and Program Director for the Leukemia and Lymphoma Program at the University of Pittsburgh Cancer Institute. I have an extensive background in the field of immunotherapy for cancer treatment, as evidenced by my Curriculum Vitae, which is attached. In particular, I have been the principal investigator on clinical trials relating to immunotherapy of various B-cell malignancies. For example, I am currently the principal investigator for a Phase II clinical trial to study effects of a combination of rituximab, fludarabine and cyclophosphamide on patients with previously untreated chronic lymphocytic leukemia.

2. I am familiar with the article Maloney *et al.*, *Blood*, 84(8): 2457-2466 (1994). This article relates to results from a Phase I clinical trial to evaluate the safety of anti-CD20 antibody as a single agent therapeutic.

3. Maloney 1994 states, on page 2465, that "extension of these studies to patients with minimal disease, using antibody alone or in combination with conventional therapies, may provide the greatest benefit. The disclosure in Maloney that anti-CD20 may be combined with a "conventional therapy" would not have suggested to me therapy with a combination of an anti-CD20 antibody and another antibody, such as an anti-CD22 antibody. This is because "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies. The current clinical trial which I referenced above in paragraph 1 is just such a combination of the anti-CD20 antibody rituximab with a conventional drug therapy (fludarabine and cyclophosphamide) that is envisioned by Maloney 1994.

4. "Conventional" means "conforming to established practice or accepted standards; traditional" (The American Heritage® Dictionary of the English Language: Fourth Edition - 2000). An investigational drug in Phase I clinical trials cannot be considered a conventional therapy, *i.e.*, it does not conform to established practice or accepted standards." By definition, investigational drugs have not been "accepted." Companies can provide investigational drugs to doctors if they are part of a drug trial covered by an FDA-approved protocol, and such drugs are by definition not conventional, since they are not available for use by any doctor on any patient.

5. In 1994 (and later) antibody therapies were not "conventional," and therefore Maloney's comment regarding the addition of "conventional therapies" to his anti-CD20 antibody suggests to a skilled clinician a combination of the anti-CD20 single antibody therapy with a chemotherapy. It would not have suggested therapy with a combination of antibodies. "Conventional therapies," circa 1994 and later, were chemotherapies. The first approved antibody for therapy of any malignancy was the anti-CD20 antibody rituximab that is the subject of Maloney, but it was not approved until 1997, and therefore there was no cancer therapy with any antibody that was a conventional therapy in 1994.

6. Treatment with anti-CD22 antibody was not conventional circa 1994. For example, Goldenberg *et al., J. Clin. Oncol.*, 9: 548-564 (1991) relates to results from a pilot Phase I study involving a small number of patients to see the feasibility of giving this radiolabeled antibody, involving targeting tumor and organs, doses delivered to tumor and normal organs, and any evidence of efficacy in a small number of patients, and does not establish that treatment with anti-CD22 antibody was "conventional." Juweid *et al. Cancer Res.*, 55:5899s-5907s (1995) is a follow-on report of further treatment of patients with low doses and

initial results of high dose therapy in a Phase I trial. Here again, these were early investigational studies and do not represent conventional therapy.

7. Current reviews and texts support the fact that antibody therapy using a combination of antibodies to different targets is not conventional. No such combination therapy has been approved, and even combinations of rituximab with conventional chemotherapy for lymphoma have only been approved by the FDA within the past two years. Although some articles began to discuss the possibility of combination antibody therapies following publication by Immunomedics of their studies of epratuzumab and rituximab in about 2002/2003, none of these indicate that such therapy is "conventional." For example, "What is New in Lymphoma," published in 2004, cites rituximab as an advancement in the treatment of NHL. Efforts to improve the activity of rituximab are noted, and include increasing the number of weekly infusions, delivering higher doses and increasing dose density. Combinations with CHOP are also mentioned. The article references both a Phase II study of a combination of rituximab with epratuzumab and a phase I/II study of the combination of galiximab and rituximab, both of which were reported in 2003, demonstrating that combination antibody therapy was still very much investigational at this later date. Cheson, CA *Cancer J Clin*, Sep-Oct; 54(5):260-72 (2004).

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I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/16/07
Date

Kenneth Foon, MD

CURRICULUM VITAE

BIOGRAPHICAL

Name:	Kenneth A. Foon, MD	**Birth Date:** 3/7/47
Home Address:	416 William Street Pittsburgh, PA 15211	**Birth Place:** Detroit, MI
Home Phone:	(412) 481-2724	**Citizenship:** USA
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Business Phone:	(412) 623-5898	**Spouse:** Rebecca A. Garrett, MD
Business Fax:	(412) 648-6579	

EDUCATION AND TRAINING

UNDERGRADUATE:

Dates Attended	Name & Location of Institution	Degree Received and Year	Major Subject
1964-1966	University of Michigan		
1966-1968	Wayne State University	BS, 1968	Biology

GRADUATE:

Dates Attended	Name & Location of Institution	Degree Received and Year	Major Subject
1968-1972	Wayne State University	M.D, 1972	Medicine

POSTGRADUATE:

Dates Attended	Name & Location of Institution	Name of Program Director and Discipline
1972-1973	University of California, San Diego School of Medicine, San Diego, CA	Nathan Zvailler, MD Straight Internal Medicine Internship
1976-1977	Washington VA & Georgetown Hospitals, District of Columbia	Hyman Zimmerman, MD Straight Internal Medicine First Year Residency
1977-1980	University of California, Los Angeles Los Angeles, CA	Martin Cline, MD Fellow in Hematology and Oncology

ACADEMIC:

Years Inclusive	Name & Location of Institution	Rank/Title
1980-1981	University of California Los Angeles School of Medicine Los Angeles, CA	Assistant Professor of Medicine (tenure track)
1985-1987	University of Michigan School of Medicine Ann Arbor, MI	Associate Professor of Medicine (with tenure)
1985-1987	University of Michigan School of Medicine Ann Arbor, MI	Associate Chief, Div. of Hematology-Oncology Director of Clinical Hematology
1987-1991	State University of New York at Buffalo Buffalo, NY	Professor, Dept. of Medicine (with tenure)
1987-1991	Roswell Park Cancer Institute Buffalo, NY	Chief, Division of Clinical Immunology
1991-1992	Department of Molecular and Experimental Medicine, The Scripps Research Institute San Diego, CA	Adjunct Member

1991-1992	Ida M. and Cecil H.Green Cancer Center Scripps Clinic and Research Foundation San Diego, CA	Associate Director for Clinical Research
1993-1999	University of Kentucky School of Medicine Lexington, KY	Chief, Div. of Hematology-Oncology Professor of Medicine (with tenure)
1993-1999	Markey Cancer Center University of Kentucky School of Medicine Lexington, KY	Director
1999-2001	University of Cincinnati School of Medicine Cincinnati, OH	Associate Director Division of Hematology-Oncology Professor of Medicine (with tenure)
1999-2001	Barrett Cancer Center University of Cincinnati School of Medicine Cincinnati, OH	Director
2001-2003	Stanford University School of Medicine Stanford, CA	Clinical Professor of Medicine
2003-2006	University of Pittsburgh Cancer Institute	Co-Leader of the Biological Therapeutics Program
2005-Present	University of Pittsburgh Cancer Institute	Interim Director of Minority Outreach
2003-Present	University of Pittsburgh Cancer Institute	Director of Clinical Investigations Co-Leader of the Hematologic Malignancies Program
2003-Present	University of Pittsburgh School of Medicine	Professor of Medicine

NON-ACADEMIC

Years Inclusive	Name & Location of Institution	Rank/Title
1973-1975	National Eye Institute, National Institutes of Health Bethesda, MD and guest worker National Institute of Dental Research, Laboratory of Microbiology and Immunology Bethesda, MD	Research Associate and Lieutenant, U.S. Public Health Service
1975-1976	National Institute of Dental Research, Laboratory of Microbiology and Immunology, National Institute of Health, Bethesda, MD	Clincial Associate and Lieutenant Commander, U.S. Public Health Service
1981-1982	Head, Monoclonal Antibody-Hybridoma Section, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute Frederick, MD	Senior Investigator and Lieutenant Commander, U.S. Public Health Service
1982-1985	Head, Clinical Investigations Section, Biologic Response Modifiers Program, Division of Cancer Treatment National Cancer Institute Frederick, MD	Senior Investigator and Lieutenant Commander, U.S. Public Health Service (tenured in 1984)
2001-2003	Abgenix Inc. Fremont, CA	Director, Clinical Development for Oncology

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CERTIFICATION AND LICENSURE

Specialty Certification:

Certifying Board	Year

National Board of Medical Examiners	1973
American Board of Internal Medicine	1978
American Board of Internal Medicine Subspecialty of Hematology	1980
American Board of Internal Medicine Subspecialty of Medical Oncology	1981

Medical Licensure:

Licensing Board/State	**Year**
California (active)	1973
Maryland (inactive)	1973
Michigan (inactive)	1982
New York (inactive)	1987
Kentucky (inactive)	1993
Ohio (inactive)	1999
Pennsylvania (Active)	2003

MEMBERSHIPS IN PROFESSIONAL AND SCIENTIFIC SOCIETIES

Organization	**Year**
American College of Physicians (Fellow)	1978-present
American Society of Hematology	1980-present
American Association for the Advancement of Sciences	1980-present
American Society of Clinical Oncology	1981-present
American Association for Cancer Research	1982-present
The Society of Biological Therapy	1982-present

Clinical Immunology Society	1987-present
Member, Leukemia Committee, Southwest Oncology Group	1987-2001
International Society for Experimental Hematology	1991
Member, Colorectal Disease Committee, National Surgical Adjuvant Breast and Bowel Project	1997-2001
Member, Melanoma Committee, Southwest Oncology Group	1997-2000
Member, Colorectal Committee, American College of Surgeons	1999-2001
Member, Lymphoma, Committee, Eastern Cooperative Oncology Group	2003-Present
Member, Leukemia Committee, ECOG	2003-Present

HONORS

Title of Award	**Year**
Alpha Omega Alpha	1971
Dr. A. Ashley Rousuck Award in Internal Medicine (Wayne State University School of Medicine)	1971
Gordon B. Myers Award in Internal Medicine (Wayne State University School of Medicine)	1972
Medical Degree Awarded with High Distinction, Wayne State University	1972
Fellow of the American College of Physicians	1982
Distinguished Alumni Award, Wayne State University School of Medicine	1987

Michigan Science Trailblazer	1988
Faculty Research Award for Excellence in Research, University of Kentucky	1998
Chairman's Award for Excellence in Research, University of Kentucky, Dept. of Internal Medicine	1998

PUBLICATIONS

1. Refereed Articles

1. **Foon KA**, Wahl SM, Oppenheim JJ and Rosenstreich DL: Serotonin-induced production of a monocyte chemotactic factor by human peripheral blood leukocytes. J Immunol 117:154-1552, 1976.

2. Sher NA, **Foon KA**, Fishman ML and Brown T: Demonstration of monocyte chemotactic factors in the aqueous humor during experimental immunogenic uveitis in the rabbit. Infect Immun 13:1110-1116, 1976.

3. Sher ND, Douglas DJ, Mindrup E, Minaii LA and **Foon KA**: Macrophage migration inhibition factor activity in the aqueous humor during experimental corneal xenograft and allograft rejection. Am J Ophthalmol 82:858-865, 1976.

4. **Foon KA**, Yuen K, Ballintine E and Rosenstreich D: Analysis of the systemic corticosteroid sensitivity of patients with primary open angle glaucoma. Am J Ophthalmol 83:167-173, 1977.

5. **Foon KA**, Naiem F, Yale C and Gale RP: Acute myelogenous leukemia: Morphologic subclass and response to therapy. Leuk Res 3:171-173, 1979.

6. **Foon KA**, Billing RJ and Terasaki PI: Dual B and T markers in acute and chronic lymphocytic leukemia. Blood 55:16-20, 1980.

7. Billing RJ, Clark BM, Koeffler P, **Foon KA** and Terasaki PI: Acute myelocytic leukemia heteroantisera. Clin Immunol Immunopathol 16:202-210, 1980.

8. **Foon KA**, Yale C, Clodfelter K and Gale RP: Effect of posttreatment hepatitis on survival of patients with acute myelogenous leukemia. JAMA, 244:1806-1807, 1980.

9. **Foon KA**, Fitchen JH, Billing RJ, Belzer MB, Terasaki PI and Cline MJ: An antithymocyte serum non-cytotoxic to myeloid progenitor cells: Candidate serum for prevention of graft-versus-host disease in bone marrow transplantation. Clin Immunol Immunopathol 16:416-422, 1980.

10. **Foon KA**, Billing RJ, Terasaki PI and Cline MJ: Immunologic classification of acute lymphocytic leukemia: Implications for normal lymphoid differentiation. Blood 56:1120-1126, 1980.

11. **Foon KA**, Herzog P, Billing R, Terasaki PI and Feig S: Immunologic classification of childhood acute lymphocytic leukemia. Cancer 47:280-284, 1981.

12. **Foon KA**, Naeim F, Saxon A, Stevens R and Gale RP: Leukemia of T-helper lymphocytes: Clinical and functional characterization. Leuk Res 5:1-10, 1981.

13. **Foon KA**, Billing RJ, Fitchen JH, Belzer MB, Drew SI and Terasaki PI: An antigen expressed by cells of the myelo-monocytic lineage. Am J Hematol 10:259-267, 1981.

14. Belzer M, Fitchen JH, Ferrone S, **Foon KA**, Billing RJ and Golde DW: Expression of HLA-DR antigen on human erythroid progenitor cells as determined by monoclonal anti-DR antibodies and heteroantiserum. Clin Immunol Immunopathol 20:111-115, 1981.

15. Gale RP, **Foon KA**, Cline MJ and Zighelboim J: Intensive chemotherapy for acute myelogenous leukemia. Ann Intern Med 94:753-757, 1981.

16. Fitchen JH, **Foon KA** and Cline MJ: The antigenic characteristics of hematopoietic stem cells. N Engl J Med 305:17-25, 1981.

17. **Foon KA**, Zighelboim J, Yale C and Gale RP: Intensive chemotherapy is the treatment of choice for elderly patients with acute myelogenous leukemia. Blood 58:467-470, 1981.

18. **Foon KA**, Filderman A and Gale RP: Histiocytic lymphoma following resolution of sarcoidosis. Med Pediatr Oncol 9:325-331, 1981.

19. Hoffman F, **Foon KA**, Smith D, Kransler J, Ciciarelli J and Billing R: Functional properties of subsets of T lymphocytes defined by special antigens. Clin Exp Immunol 44:476-484, 1981.

20. Hocking WG, Billing R.J, **Foon KA** and Golde DW: Human alveolar macrophages express DR antigens. Blood 58:1040-1042, 1981.

21. Linker-Israeli M, Billing RJ, **Foon KA** and Terasaki PI: Monoclonal antibodies reactive with acute myelogenous leukemia cells. J Immunol 127:2473-2477, 1981.

22. Billing R, Terasaki PI, Sugich L and **Foon KA**: Detection of differentiation antigens by use of monoclonal antibodies. J Immunol Methods 47:289-294, 1981.

23. Schroff RW, **Foon KA**, Billing RJ and Fahey JL: Immunologic classification of lymphocytic leukemias based on monoclonal antibody-defined cell surface antigens. Blood 59:207-215, 1982.

24. **Foon KA** and Haskell CM: Inadvertent overdose with lomustine (CCNU) followed by hematologic recovery. Cancer Treat Reports 66:1241-1241, 1982.

25. **Foon KA** and Gale RP: Controversies in the therapy of acute myelogenous leukemia. Am J Med 72:963-979, 1982.

26. **Foon KA**, Schroff RW and Gale RP: Cell surface markers on leukemia and lymphoma cells: Recent advances. Blood 60:1-19, 1982.

27. Billing RJ, **Foon KA** and Linker-Israeli M: The immunological classification of leukemia based on a rapid microcytoxicity test. Clin Exp Immunol 49:142-148, 1982.

28. Schroff RW and **Foon KA**: Heterogeneity in a lymphoid tumor: Co-expression of T and B surface markers. Blood 60:373-380, 1982.

29. **Foon KA**, Schroff RW and Fliegel S: Monoclonal antibody characterization of plasmacytoma cells associated with T lymphocytes with a suppressor phenotype. Clin Immunol Immunopathol 25:59-66, 1982.

30. **Foon KA**, Bernhard MI and Oldham RK: Monoclonal antibody therapy: Assessment by animal tumor models. J Biol Response Mod 1:277-304, 1982.

31. Sherwin SA, Knost JA, Fein S, Abrams PG, **Foon KA**, Ochs JJ, Schoenberger C, Maluish AE and Oldham RK: A multiple dose phase I trial of recombinant leukocyte A interferon in cancer patients. JAMA 248:2461-2466, 1982.

32. Key ME, Bernhard MI, Hoyer LC, **Foon KA**, Oldham RK and Hanna MG: Guinea pig line 10 hepatocarcinoma model for monoclonal antibody serotherapy: *In vivo* localization of a monoclonal antibody in normal and malignant tissues. J Immunol 120:1451-1457, 1983.

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34. Bernhard MI, **Foon KA**, Oeltmann TN, Key ME, Hwang KM, Clarke CG, Christensen WL, Hoyer LC, Hanna MG and Oldham RK: Guinea pig line 10 hepatocarcinoma model:

Characterization of monoclonal antibody and *in vivo* effect of unconjugated antibody and antibody conjugated to diptheria toxin A chain. Cancer Res 43:4420-4428, 1983.

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36. **Foon KA**, Smalley RV, Riggs CW and Gale RP: The role of immunotherapy in acute myelogenous leukemia. Arch Intern Med 143:1726-1734, 1983.

37. Knost JA, Sherwin SA, Abrams PG, Ochs JJ, **Foon KA**, Williams R, Tuttle R and Oldham RK: The treatment of cancer patients with human lymphoblastoid interferon: A comparison of two routes of administration. Cancer Immunol Immunother 15:148-155, 1983.

38. Abrams PG, Knost JA, Clarke G, Wilburn S, Oldham RK and **Foon KA**: Determination of the optimal human cell lines for development of human hybridomas. J Immunol 131:1201-1204, 1983.

39. **Foon KA**, Buescher S, Kimball ES, Huang LC, Stevenson HC, Clarke G, Gregoria T and Harley JB: Monoclonal antibody to human eosinophils recognizing 95 kD surface membrane antigen. Hybridoma 2:393-402, 1983.

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41. Hwang JM, **Foon KA**, Cheung PH, Pearson JW and Oldham RK: Selective antitumor effect on L10 hepatocarcinoma cells of a potent immunoconjugate composed of the A chain of abrin and a monoclonal antibody to a hepatoma-associated antigen. Cancer Res 44:4578-4586, 1984.

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43 Oldham RK, Morgan AC, Woodhouse CS, Schroff RW, Abrams PG and **Foon KA**: Monoclonal antibodies in the treatment of cancer: Preliminary observations and future prospects. Med Oncol Tumor Pharmacother 1:51-62, 1984.

44. Abrams PG, Ochs JJ, Giardina SL, Morgan AC, Wilburn SB, Wilt AR, Oldham RK and **Foon KA**: Production of large quantities of human immunoglobulin in the ascites of athymic mice: Implication for the development of anti-human idiotype monoclonal antibodies. J Immunol 132:1611-1613, 1984.

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47. Champlin R, Jacobs A, Gale RP, Boccia R, Elashoff R, **Foon KA** and Zighelboim J: Prolonged survival in acute myelogenous leukemia without maintenance chemotherapy. Lancet 1:894-896, 1984.

48. Schroff RW, Farrell MM, Klein RA, Oldham RK and **Foon KA**: T65 antigen modulation in a phase I monoclonal antibody trial with chronic lymphocytic leukemia patients. J Immunol 133:1641-1648, 1984.

49. **Foon KA**, Neubauer RH, Wikstrand CJ, Schroff RW, Rabin H and Seeger RC: Human Thy-1 antigen: Distribution on human and non-human primate hematopoietic cells. J Immunogenet 11:233-244, 1984.

50. **Foon KA**, Schroff RW, Bunn RA, Mayer D, Abrams PG, Fer MF, Ochs JJ, Bottino GC, Sherwin SA, Herberman RB and Oldham RK: Effects of monoclonal antibody therapy in patients with chronic lymphocytic leukemia. Blood 64:1085-1094, 1984.

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52. Reynolds CW and **Foon KA**: T-lymphoproliferative disease and related disorders in man and experimental animals. A review of the clinical, cellular and functional characteristics. Blood 64:1146-1158, 1984.

53. Fer MF, Bottino GC, Sherwin SA, Hainesworth JD, Abrams PG, **Foon KA** and Oldham, RK: Atypical tumor lysis syndrome in a patient with T-cell lymphoma following recombinant interferon therapy. Am J Med 77:953-956, 1984.

54. Bunn PA, **Foon KA**, Idhe DC, Winkler CF, Zeffren J, Sherwin SA and Oldham RK: Recombinant leukocyte A interferon: An active agent in advanced cutaneous T-cell lymphoma. Ann Intern Med 101:484-487, 1984.

55. Stevenson HC, Kimball ES, Buescher S, Clarke G and **Foon KA**: Monoclonal antibody to human monocytes and granulocytes: Isolation of membrane antigens and lack of effect on leukocyte functions *in vitro*. Hybridoma 3:247-261, 1984.

56. **Foon KA**, Sherwin SA, Abrams PG, Longo DL, Fer MF, Stevenson HC, Ochs JJ, Bottino GC, Schoenberger CS, Zeffren J, Jaffe ES and Oldham RK: Treatment of advanced non-Hodgkin's lymphoma with recombinant leukocyte A interferon. N Engl J Med 311:1148-1152, 1984.

57. Morgan AC, Woodhouse CS, Knost JA, Abrams PG, Clarke GC, Arthur LO, McIntrye R, Ochs JJ, **Foon KA**, Hanna MG and Oldham RK: Monoclonal antibodies to human colorectal tumor-associated antigens: Improved elicitation and subclass restriction. Hybridoma 3:233-245, 1984.

58. Sherwin SA, **Foon KA**, Oldham RK, Abrams PG, Heyman MR, Ochs JJ, Watson R and Maluish A: A preliminary phase I trial of partially purified interferon-gamma (IFN-gamma) in patients with disseminated cancer. Biol Response Mod 3:599-607, 1984.

59. Bunn PA, Carrasquillo JA, Keenan AM, Schroff RW, **Foon KA**, Hsu SM, Gazdar AF, Reynolds JJ, Perentesis P and Larson SM: Imaging of T-cell lymphoma by radiolabeled monoclonal antibody. Lancet 1219-1221, 1984.

60. Wei N and **Foon KA**: Sclerodactyly in a patient with mycosis fungoides. Arch Intern Med 145:139-140 1985.

61. Schroff RW, **Foon KA**, Wilburn SB, Oldham RK and Morgan AC: Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 45:879-885, 1985.

62. Schroff RW, Woodhouse CS, **Foon KA**, Farrell MM, Klein RA, Oldham RK and Morgan AC: Intratumor localization of monoclonal antibody in patients with melanoma treated with antibody to a 250,000-dalton melanoma-associated antigen. J Natl Cancer Inst 74:299-306, 1985.

63. **Foon KA**, Bottino GC, Abrams PG, Fer MF, Longo DL, Schoenberger CS and Oldham RK: A phase II trial of recombinant leukocyte A interferon for patients with advanced chronic lymphocytic leukemia. Am J Med 78:216-220, 1985.

64. Gale RP and **Foon KA**: Chronic lymphocytic leukemia: Recent advances in biology and treatment. Ann Intern Med 103:101-120, 1985.

65. Giardina SL, Schroff RW, Woodhouse CS, Golde DW, Oldham RK, Cleary ML, Sklar J, Pritikin N and **Foon KA**: Detection of two malignant B-cell clones in a single patient using anti-idiotype monoclonal antibodies and immunoglobulin gene rearrangement. Blood 66:1017-1021, 1985.

66. Giardina SL, Schroff RW, Kipps TJ, Woodhouse CS, Abrams PG, Rager HC, Morgan AC and **Foon KA**: The generation of monoclonal anti-idiotype antibodies to human B-cell derived leukemias and lymphomas. J Immunol 135:653-658, 1985.

67. **Foon KA**, Rossio JL, Schroff RW, Wahl SM, Abrams PG, Rager HC, Pickeral SF and Fidler IJ: The generation of stable human T-cell hybridomas which constitutively produce interleukin-2 and chemotactic factor. Hybridoma 4:211-222, 1985.

68. Stevenson HC, Abrams PG, Schoenberger CS, Smalley RV, Herberman RB and **Foon KA**: A phase I evaluation of poly ICLC in cancer patients. J Biol Response Mod 4:650-655, 1985.

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72. Bunn PA Jr, Ihde DC and **Foon KA**: The role of recombinant leukocyte A interferon in the therapy of cutaneous T-cell lymphomas. Cancer 57:1315-1321, 1986.

73. **Foon KA** and Todd RF III: Immunologic classification of leukemia and lymphoma. Blood 68:1-31, 1986.

74. Giardina SL, **Foon KA**, Beatty SM and Morgan AC Jr: Evaluation of alternative methodologies for the generation of monoclonal anti-idiotypic antibodies to human B-cell derived leukemias and lymphomas. J Immunol Methods 89:1-7, 1986.

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76. **Foon KA**, Nakano GM, Koller CA, Longo DL and Steis RG: Response to 2'-deoxycoformycin after failure of interferon alpha in non-splenectomized patients with hairy cell leukemia. Blood 68:297-300, 1986.

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83. Stevenson HC, Keenan AM, Woodhouse C, Ottow RT, Miller P, Stellar EP, **Foon KA**, Abrams PG, Beman J, Larsen SM and Sugarbaker PH: Fate of gamma-interferon-activated killer blood monocytes adoptively transferred into the abdominal cavity of patients with peritoneal carcinomatosus. Cancer Res 47:6100-6103, 1987.

84. Keenan AM, Weinstein JN, Carrasquillo JA, Bunn PA Jr, Reynolds JC, **Foon KA**, et al: Immunolymphoscintigraphy and the dose-dependence of Indium^{111} labeled T-101 monoclonal antibody in patients with cutaneous T-cell lymphoma. Cancer Res 47:6093-6097, 1987.

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SK. DNA vaccines based on the structure of an anti-idiotype antibody (3H1) mimicking carcinoembryonic antigen (CEA). *Proc. Amer. Assoc. Cancer Res.* 40:323, 1999.

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155. M.A. Melan, **K.A. Foon**, S.M. Marks, and J.A. Kant. Immunoglobulin Havey Chain (IgH) Gene Somatic Hypermutation Status Using Homoduplex-Separated RT-PCR Products. American Society of Hematology 46th Annual Meeting program and Abstracts, Blood 104:11; Pt 2 of 2 Pts, 2004.

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157. N.A. DeMonaco, M. Wu, J. Osborn, T. Evans, **K.A. Foon**, S.H. Swerdlow, J.A. Kant, J. Joyce, S. Land, and S.A. Jacobs. Phase II trial of abbreviated CHOP-Rituximabfollowed by ^{90}Y Ibritumomab Tiuxetan (Zebalin) and Rituximab in Patientswith Previously-Untreated Follicular Non-Hodgkin Lymphoma (NHL). American Society of Hematology 47th Annual Meeting program and Abstracts, Blood 106:11; Pt 1 of 2 Pts, 2005.

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160. A. A. Tarhini, S. Land, F. Lim, G. J. Kiefer, L. Pietragallo, R. A. Pinkerton, M. Sulecki, D. Meisner, P. M Schaefer, **K.A. Foon**. Early results of modified fludarabine, cyclophosphamide, and rituximab (mFCR) for patients with previously untreated advanced chronic lymphocytic leukemia (CLL). 2006 ASCO Annual Meeting Proceedings; J Clin Onc, Vol. 24, No. 18S Part I of II, 2006.

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4. Other Publications

1. **Foon KA** and Gale RP: Post-transfusion hepatitis in acute myelogenous leukemia. JAMA 246:216, 1981 (letter)

2. **Foon KA**, Zighelboim J and Gale RP: Treatment of acute myelogenous leukemia in older patients. N Engl J Med 305:1470, 1981. (letter)

3. **Foon KA**: Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med 307:686, 1982. (letter)

4. Bernhard MI, **Foon KA**, Oldham RK and Keenan A: The importance of traditional methods of measuring tracer biodistributions. Cancer Res 44:2731-2732, 1984. (letter)

5. **Foon KA** and Dougher G: Increased growth of eyelashes in a patient given leukocyte A interferon. N Engl J Med 311:1259, 1984. (letter)

6. **Foon KA** and Schroff RW: The uses of monoclonal antibodies in the diagnosis and treatment of leukemia and lymphoma. In: *In Vitro Monograpy*, No. 5. 1984, pp 169-188.

7. Abrams PG, McClamrock EA and **Foon KA**: Evening administration of alpha interferon. N Engl J Med 312:443-444, 1985. (letter)

8. Reynolds CW, **Foon KA** and Herberman RB: Terminology in Tγ lymphoprolifertive disease. Blood 66:248-249, 1985. (letter)

9. **Foon KA**: Interferon-alpha: The next decade. Interactions 1:3, 1986. (editorial)

10. **Foon KA:** Interferons. Hoffman La Roche, 1986. (video)

11. **Foon KA**: Biological therapy of chronic myelogenous leukemia. Oncology 1:49-52 1987. (editorial)

12. **Foon KA**: Application of monoclonal antibodies in the diagnosis and therapy of cancer. NeoRx Pharm Inc, 1988, pp 1-27. (monograph)

13. **Foon KA:** Antibody Structure and Function. NeoRx Pharm Inc, 1988. (video).

14. **Foon KA:** Therapy of Acute Leukemia: Recent Progress and Future Directions. Wyeth Labortories, 1988. (video)

15. **Foon KA**, Champlin RE and Gale RE: Therapy of acute lymphoblastic leukemia. UCLA Symposium. In: *Acute Lymphoblastic Leukemia*, Vol. 108., pp 157-196, 1990.

16. Vaickus L and **Foon KA**: Clinical immunology at Roswell Park Cancer Institute. Biotherapy of cancer. Oncoline, pp 3-4, 1990. (editorial)

17. Foon **KA**, Gale RP: Is there a T-cell form of chronic lymphocytic leukemia. Leukemia 6:867-8, 1992. Leukemia 7:916-8, 1993. (letter)

18. **Foon KA:** Cancer Vaccine Therapy: New Approaches, Video Journal of Oncology, Secaucus, NJ 1993. (video)

19. **Foon KA** and Bhattacharya-Chatterjee M. Idiotype vaccines in the clinic. Nature Med 4:870, 1998. (letter)

TEACHING

Biotherapy: New Opportunities for Cancer Treatment, Grand Rounds, Scripps Clinic and Research Foundation, La Jolla, CA, February 28, 1992.

Acute Leukemia, Internal Medicine Residency Program, University of Kentucky Medical Center, Lexington, KY, August 16, 1993.

Hodgkin's Disease, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, September 10, 1993.

Biologic Therapy and Growth Factors, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, October 8, 1993.
Anti-Idiotype Immunotherapy in Cancer Patients, Lecture-Division of Clinical Chemistry, University of Kentucky Hospital, Lexington, KY, April 27, 1994.

Chronic Lymphocytic Leukemia, Internal Medicine Grand Rounds, University of Kentucky Medical Center, Lexington, KY, July 6, 1994.

Acute Leukemia, Internal Medicine Residency Program, University of Kentucky Medical Center, Lexington, KY, August 16, 1994.

Anti-Idiotype Vaccine Therapy of CEA positive tumors, Internal Medicine Research Seminar, University of Kentucky Medical Center, Lexington, KY, September 8, 1994.

Melanoma, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, February 3, 1995.

Lymphoma, Internal Medicine Residency Program, University of Kentucky Medical Center, Lexington, KY, March 23, 1995.

Chronic Lymphocytic Leukemia, Internal Medicine Residency Program, University of Kentucky Medical Center, Lexington, KY, April 28, 1995.

Overview of the Cancer Center, Health Administration, University of Kentucky Medical Center, June 22, 1995.

Non-Hodgkin's Lymphoma, Internal Medicine Residency Program, University of Kentucky Medical Center, Lexington, KY, September 11, 1995.

Hodgkin's Disease, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, September 15, 1995.

Non-Hodgkin's Lymphoma, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, September 29, 1995.

Chronic Lymphoid Leukemias, Hematology-oncology grand Rounds, Confernce, Univeristy of Cincinnati, Cincinnati, OH, September 28, 2000

Colon Cancer, Internal Medicine residency Program, University of Cincinnati, Cincinnati, OH, September 27, 2000

Non-Hodgkin's Lymphoma, Internal Medicine residency Program, University of Cincinnati, Cincinnati, OH, June 26, 2000

Melanoma, Internal Medicine Residency Program, University of Cincinnati, Cincinnati, OH, April 14, 2000.

Lymphoma, Internal Medicine Residency Program, University of Cincinnati, Cincinnati, OH, March 13, 2000.

Anti-Idiotype Cancer Vaccines, Children's Hospital Medical Center Research Foundation, Basic Sciences, Cincinnati, OH, January 31, 2000.

Cancer Research, Division of Pharmacology, Ph.D. Program, University of Cincinnati, OH, January 7, 2000.

Lymphomas, Internal Medicine Residency Program, University of Cincinnati, Cincinnati, OH, January 6, 2000.

Melanoma, Internal Medicine Residents, University of Cincinnati Hospital, June 24, 1999.

Anti-Idiotype Cancer Vaccines, Children's Hospital Medical Center Research Foundation, Basic Scientists, January 31, 2000.

Cancer Research, Division of Pharmacology, Ph.D. Program, University of Cincinnati College of Medicine, January, 7, 2000.

Lymphomas, Internal Medicine Residents, University of Cincinnati Hospital, January 6, 2000

Melanoma, Internal Medicine Residents, Univeristy of Cincinnati Hospital, Cincinnati, OH, December 9, 1999.

Lymphomas, Internal Medicine Residents, University of Cincinnati Hospital, October 4, 1999.

Lymphomas, Internal Medicine Residents, University of Cincinnati Hospital, September 14, 1999.

A Novel Immunotherapeutic Approach for the Adjuvant Treatment of Colon Cancer, Internal Medicine Grand Rounds, University of Kentucky Medical Center, Lexington, KY, September 4, 1996.

Melanoma, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, August 16, 1996.

Acute Leukemia, Internal Medicine Residency Program, University of Kentucky Medical Center, Lexington, KY, August 8, 1996.

Low Grade Lymphomas, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, June 28, 1996.

Problem Based Learning Tutor Session, Internal Medicine, University of Kentucky Medical Center, April 1-15, 1996.

Leukemia and Lymphoma, Physician Assistants Lecture, University of Kentucky Medical Center, March 7, 1996.

Acute Leukemias, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, February 2, 1996.

Non-Hodgkin's Lymphoma, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, September 29, 1995.

Hodgkin's Disease, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, September 15, 1995.

Low Grade Lymphomas, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, June 28, 1996.

Problem Based Learning Tutor Session, Internal Medicine, University of Kentucky Medical Center, April 1-15, 1996.

Leukemia and Lymphoma, Physician Assistants Lecture, University of Kentucky Medical Center, March 7, 1996.

Acute Leukemias, Hematology/Oncology Fellows Board Review Lecture Series, University of Kentucky Medical Center, February 2, 1996.

Grand Rounds, University of Cincinnati Hospital, Cincinnati, OH, 2004.

Medical Grand Rounds, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, 2004

Presentations for Lymphoma/Leukemia Tumor Board, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, 2003-2006.

Presentations for Hematologic Malignancies Program Seminar Series, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, 2003-2006.

Presentations for Biologic Therapeutics Committee, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, 2003-2006.

Presentations for Hematologic Malignancy Steering Committee, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, 2003-2006.

Nipent® (pentostatin) in CLL, Supergen Nipent® Strategic Planning Community Advisory Board, Detroit, Michigan, August 6, 2005.

Recent Advances in the Biology and Treatment of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Supergen, January 14, 2005

Leukemia and Lymphoma, Houseteaching Staff Lecture Series for Residents, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, July 17, 2003; October 24, 2003; and November 7, 2003.

Leukemia and Lymphoma, Houseteaching Staff Lecture Series for Residents, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, February 24, 2004; April 1, 2004; May 10, 2004, and June 22, 2004.

Leukemia and Lymphoma, Houseteaching Staff Lecture Series for Residents, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, January 14, 2005; February 15, 2005; March 29, 2005; May 23, 2005; July 21, 2005.

Monoclonal Antibodies and The Paradigm Shift in the Treatment of B-Cell Lymphomas and Leukemias, Hematology/Oncology Fellows Lecture Series, University of Pittsburgh School of Medicine/Department of Hematology/Oncology, June 17, 2005.

Recent Advances in the Biology and Treatment of Low Grade Lymphomas, Lecture Series for Hematology/Oncology Fellows, University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology; May 5, 2006.

Leukemia and Lymphoma, Houseteaching Staff Lecture Series for Residents, University of Pittsburgh School of Medicine/Department of Hematology/Oncology, March 2, 2006; May 1, 2006; June 8, 2006; July 18, 2006.

Recent Advances in the Biology and Treatment of Low Grade Lymphomas "Overview of Lymphoma" as part of Neoplasia Course; Facilitator for Case 4 "Erythocytosis" of PBL cases; January 2005.

"Recent Advances and Clinical Aspects in the Treatment of CLL," sponsored by Georgia Cancer Foundation, Atlanta, GA, March 23, 2006

Current Therapeutic Modalities in the Management of CLL, Cleveland, OH, April 20, 2006.

Mentored Jennifer Larson, 4th year Medical student, and assisted w/her poster presentation, summer 2005.

Mentored Christopher Marsh, MD, resident, and assisted him w/his presentation, spring 2006.

"Recent Advances and Clinical Aspects in the Treatment of CLL/SLL," Hematology/Oncology Fellows CME Lecture, University of Pittsburgh School of Medicine/Department of Hematology/Oncology, Sponsored by Berlex Pharmaceuticals, Soba Restaurant, Shadyside, Pittsburgh, PA, September 12, 2006.

"Recent Advances and Clinical Aspects in the Treatment of CLL/SLL," Oncology Grand Rounds, USC Keck School of Medicine, Los Angeles, California, sponsored by CBCE Speakers Corp., September 22, 2006.

"Clinical Care of Lymphoproliferative Disorders," Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, part of Hematology Course for 4th year Medical School Students, January 11, 2007.

RESEARCH:

1. Current Grant Support:

Grant Number (Funded)	**Grant Title**	**Role in Project & Effort (in cal. Mths.)**		**Years Inclusive**	**Source $ Amount**
NIH RO1 CA72018	Ganglioside GD2 as Target for Immunotherapy in Melanoma	PI	3.0	8/01/05-8/30/09	$1,250, 000 (direct)

C-Change	A Statewide Cancer Clinical Trials Network in Pennsylvania	PI	1.8	7/1/05- 12/31/07	$200,000 (direct)
Genentech	Fludarabine, cyclo-phosphamine and rituximab for untreated patients with chronic lymphocytic leukemia	PI	9.0	9/1/04 – 9/30/06	$103,750 (direct)

2. Prior Grant Support

Louis Sklarow Memorial Fund, "Monoclonal Antibodies for B-Cell Lymphomas," Kenneth A. Foon, M.D., Principal Investigator, 7/1/86 - 6/31/87, $42,000 (total cost).

NIH 1 RO1 CA43212-01, "Mechanisms of Interferon Action in Hairy-Cell Leukemia," Kenneth A. Foon, M.D., Principal Investigator, 9/30/86 - 6/30/89, $173,960 (total direct cost).

NIH PO1 CA42768, "Radiopharmaceutical Diagnosis and Treatment of Cancer," Subproject: "Preclinical and Clinical Treatment with Monoclonal Antibodies to B-Cell Lymphomas," Kenneth A. Foon, M.D., Principal Investigator, 9/30/87 - 2/28/89, $265,803 (total direct cost).

"A Phase I/II Study of High-Dose, Continuous-Infusion Recombinant Human Interleukin-2 with Non-Small Cell Lung Cancer and Resistant Lymphoma," Kenneth A. Foon, M.D., Principal Investigator, Hoffmann La Roche, Inc., 10/1/88 - 9/30/89, $50,000 (total cost).

"A Phase I/II Clinical Investigation to Evaluate the Safety and Efficacy of Continuous Infusions of Recombinant Interleukin-2 and Phenylalanine Methyl Ester Pretreated Cells in Patients with Unresectable and/or Metastatic Melanoma and Renal Cell Cancer," Kenneth A. Foon, M.D., Principal Investigator, Dupont Corporation, 1/1/89 - 12/31/90, $270,000 (total cost).

NIH RO1 CA47860, "Idiotype Approach to Therapy of Human T-Cell Leukemia," Malaya Chatterjee, Ph.D., Principal Investigator, Kenneth A. Foon, M.D., Co-Investigator (10%), 4/1/89 - 3/31/92, $381,495 (total direct cost).

"Therapy of Renal Cell Carcinoma and Malignant Melanoma with Interleukin-2," Kenneth A. Foon, M.D., Principal Investigator (5%), Hoffmann La Roche, Inc., 6/1/90 - 5/31/91, $39,000 (total cost).

NIH 1PO1 CA4767-03 (Consortium), "Monoclonal Antibody Therapy of Breast Cancer," R. Ceriani, M.D., Program Director; "^{111}Indium-Labeled Monoclonal Antibody Imaging of

Metastatic Breast Cancer," Kenneth A. Foon, M.D., Principal Investigator (5%), 1/2/91 - 8/31/91, $96,025 (total direct cost).

Buffalo Foundation Grant 857-0484A, "Feasibility Study of Anti-Idiotype Monoclonal Antibody Therapy for Patients with Cutaneous T-Cell Lymphoma," Kenneth A. Foon, M.D., Principal Investigator (5%), 3/1/91 - 2/29/92, $7,000 (total cost).

NIH 1PO1 CA58880-01A2 (Program Project Grant), "Monoclonal Antibody Therapy for GI Cancer," Kenneth A. Foon, M.D., Principal Investigator (20%), 9/1/91 - 8/31/94, $900,000 (total direct cost).

NIH RO1 CA54321-01 (Consortium), "Structure-Function of Tumor-Anti-Idiotype Antibodies," Heinz Köhler, M.D., Ph.D., Principal Investigator; "Generation of Tumor-Anti-Idiotypic Antibodies," Malaya Chatterjee, Ph.D., Principal Investigator, Kenneth A. Foon, M.D., Co-Investigator (10%), 7/1/91 - 6/30/94, $130,902 (total direct cost).

Share Foundation, "Phase Ib Study of Monoclonal Anti-Idiotype Antibody Therapy for Patients with Metastatic Melanoma," 1/1/93 - 12/31/95, $125,000 (total cost).
Ortho Pharmaceutical Corp. - Treatment of previously untreated chronic lymphocytic leukemia, 1994-95, $50,000.

Tobacco & Health, Anti-Idiotype Vaccine for Human Small Cell Lung Carcinoma, 7/1/94-6/30/95, $79,900 (total direct cost).

NCI 1PO1 CA57165-04 (Program Project Grant), "Monoclonal Antibody Therapy of GI Cancer", Kenneth A. Foon, M.D., Principal Investigator. Project 1. "Generation of Anti-Idiotype Tumor Vaccines", M. Chatterjee, Ph.D., Principal Investigator. 9/30/91 - 8/31/95, $768,027 (direct cost), $126,221 (indirect cost).

Berlex, Treatment of previously untreated low grade follicular lymphoma, Kenneth A. Foon, M.D., Principal Investigator, 1994-95, $51,000.

NCI 5U10 CA46136-07, Southwest Oncology Group Clinical Study, Kenneth A. Foon, M.D., Principal Investigator, 1/1/88 - 12/31/97, $507,164 (direct cost), $244,266 (indirect cost).

NIH R01 CA60000-02, Anti-Idiotype Vaccine for Breast Cancer, Kenneth A. Foon, M.D., Principal Investigator, 12/1/94 - 11/30/97, $477,406 (total direct cost), $233,929 (indirect cost).

P20-, Planning Grants for Prospective Cancer Centers, Kenneth A. Foon, M.D., Principal Investigator, 8/1/95 - 7/30/97, $350,000 (direct cost), $171,500 (indirect cost).

NIH NCI R01 CA72773-03, "Immunotherapy of Cancer with Anti-Id Based DNA Vaccines", Principal Investigator, Sunil K. Chatterjee, Ph.D., 10/01/99- 12/31/99.

Lucille P. Markey Charitable Trust, Research Program Grant, Kenneth A. Foon, M.D., Principal Investigator, 1/2/95 - 2/15/99, $1,900,000 (total direct cost).

NIH R03 CA79401-01, Anti-Idiotype Vaccine with IL-2 for Advanced Melanoma, Kenneth A. Foon, M.D., Principal Investigator,11/1/98 - 10/30/00, $145,646 (total direct and indirect cost).

Amgen, High Grade Lymphoma, Kenneth A. Foon, M.D., Principal Investigator, 2/1/95 - 2/1/99, $15,000 ($11,910 direct costs, $3,090 indirect cost).

NIH R01 CA72018-01, Ganglioside GD2 as Target for Immunotherapy in Melanoma, Kenneth A. Foon, M.D., Co-Investigator, 8/1/96 - 6/30/01, $1,142.854 (direct cost), $1,679,995 (total cost).

Titan Pharmaceutical, Inc., Anti-Idiotype Antibody Vaccines, Kenneth A. Foon, M.D., Co-Principal Investigator, 7/1/96 - 6/30/01, $1,750,000 (total direct cost).

NCI R03 CA72468-01, Comparison of Alum and QS-21 Based Anti-Idiotype Vaccines, Kenneth A. Foon, M.D., Principal Investigator, 12/1/96 - 11/30/98, $147,000 (direct and indirect cost).

NCI U01 CA65748-01, New Therapeutic Approaches to Breast Cancer, Kenneth A. Foon, M.D., Principal Investigator, 12/1/94 - 11/30/98, $842,019 (direct cost), $412,589 (indirect cost).

NIH NCI U01CA65748, "New Therapeutic Approaches to Breast Cancer", Principal Investigator, Kenneth A. Foon, M.D., 1/1/95 – 12/31/99.

NIH NCI R03 CA68629, "Comparison of Alum and QS-21 Based Anti-Id Vaccione", Principal Investigator Kenneth A. Foon, M.D., 11/01/96 – 10/31/99

NIH RO1 CA80968-01, "Anti-Idiotype Antibody Vaccine Therapy of Human Colorectal Cancer," Consultant, Kenneth A. Foon, MD, 9/1/99 - 7/31/03.

2. Seminars and invited lectureships related to your research. (Five years)

Anti-Idiotype Cancer Vaccines, The Children's Hospital Research Foundation Immunotherapy Conference, Cincinnati, OH, January 31, 2000.

Anti-Idiotype Antibodies Directed Against Gangliosides. Melanoma at the Millennium Conference, Phoenix, AZ, February 17, 2000

Anti-idiotype Vaccine that Mimics CEA: Novel therapeutic approach to colon cancer treatment. Case Western University, Ireland Cancer Center, Blood Club Seminar, Cleveland, OH, February 25, 2000.

Clinical and Immune Responses in Resected Colon Cancer patients Treated with Anti-Idiotype Monoclonal Antibody Vaccine that mimics the CarcinoembryonicAntigen. International Conference on Advances in Cancer Immunotherapy, Princeton, NJ, March 2 - 4, 2000.

Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. 2nd Annual Walker's Cay Colloquium, Albert B. Sabin Vaccine Institute, Abaco, Bahamas, March 8 – 12, 2000.

The Barrett Cancer Center in the New Millennium. UC Board of Trustees, University of Cincinnati, Cincinnati, OH March 29, 2000.

Clinical Trials of Immunotherapeutics and Immunologic Monitoring. American Association for Cancer Research, April 3 – 4, 2000.

Targeted Therapies in the Treatment of Lymphoma, University of Louisville, Louisville, KY, Anti-idiotype Vaccine that Mimics the Carcinoembryonic Antigen. Dept. of Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, OH April 20, 2000.

Cancer Vaccines. Sabin 40th Annual Anniversary Celebration, University of Cincinnati, Cincinnati, OH April 28, 2000.

The Barrett Cancer Center and the New Millennium. UC Medical Center Orientation, Cincinnati, OH May 4, 2000.

Non-Hodgkin's Lymphoma. Miami Valley Hospital Tumor Board, Dayton, OH May 5, 2000.

Colorectal Cancer: Molecular Genetics and Therapeutic Advances. University-Wide Clinical Pathology Conference, Cincinnati, OH May 10, 2000.

Cancer Vaccines. 2000 American Society of Clinical Oncology, New Orleans, LA. May 19 – 22, 2000

Vaccine Therapies of Malignant Melanoma. Hematology-Oncology Grand Rounds, University of Cincinnati, Cincinnati, OH. May 26, 2000.

Welcome and Introduction, Cancer Survivor's Day, University of Cincinnati, Cincinnati, OH, June 4, 2000.

Vaccine Approaches to the Adjuvant Treatment of Colorectal Cancer. The Barrett Cancer Center and University of Cincinnati Annual Cancer Conference. Cincinnati, OH June 17, 2000.

Chronic Lymphoid Leukemias. Hematology-Oncology Grand Rounds Conference, University of Cincinnati, Cincinnati, OH. September 29, 2000.

Overview of Immune Flow Cytometry of Leukemias and Lymphomas. The Barrett Cancer Center, University of Cincinnati College of Medicine, Cincinnati, OH. October 7, 2000.

An Update on Cancer Vaccines. 12th Annual Western North Carolina Cancer Conferennce, Asheville, North Carolina, October 27, 2000.

Rubitecan: An Effective New Therapy in Pancreatic Cancer. Chemotherapy Foundation Symposium XVIII, New York City, New York, November 8, 2000.

Vaccine in the Treatment of GI Malignancies. Gastrointestinal Cancer Research Conference 2000, Orlando, Florida, November 16 – 18, 2000.

Anti-Idiotype Antibody that Mimics Carcinoembryonic Antigen: Novel new Approach to Colon Cancer Immunotherapy. The Molecular Medicine of Colorectal Cancer, Keystone Symposia on Molecular and Cellular Biology, Taos, New Mexico, February 1 – 2, 2001.

Expanding Options in the Treatment of Non-Hodgkin's Lymphoma". Medical City Tumor Conference, Medical City Dallas Hospital, Dallas, TX. March 21, 2001.

Monoclonal Antibodies in Combination with Chemotherapy for the Treatment of Non-Hodgkin's Lymphoma. IDEC Pharmaceuticals Corporation, Dallas, TX. March 21, 2001.

Clinical Results: A Fully Human Anti-EGFr Antibody In Patients With Advanced Cancer: Molecular Targets and Cancer Therapeutics, AACR-NCI-EORTC International Conference. October 29 – November 2, 2001

Effects of ABX-EGF, A Fully Human Anti-EGFr Antibody, in Patients with Advanced Cancer. Cancer Research Institute Antibodies 2002 Symposium: New York, NY, March 19 – 20, 2002

Current Status of Vaccine Therapy in Malignancies. Moving Beyond The Ordinary; Fourth Annual Palm Beach Cancer Symposium, Delray Beach, FL., March 22-23, 2002.

ABX-EGF, A Fully Human Anti-Epidermal Growth Factor Receptor (EGFr) Monoclonal Antibody (MAb) In Patients With Advanced Cancer. Phase 1 Clinical Trials: American Society of Clinical Oncology: Gaylord Palms Resort and Convention Center, Kissimmee, FL. May 19 – 21, 2002.

ABX-EGF, A Fully Human Anti-Epidermal Growth Factor Receptor (EGFr) Monoclonal Antibody (MAb) for the Treatment of Patients with a Variety of EGFr Positive Malignancies. Anti-Cancer Drug Discovery & Development Summit 2002: Princeton, NJ, June 17 – 19, 2002.

ABX-EGF, A Fully Human Anti-EGFR Monoclonal Antibody For The Treatment Of Advanced Caner. First International Congress on Targeted Therapies: Washington, DC, August 16-18, 2002.

Monoclonal Antibodies/Other Immune Directed Strategies In Cancer Management. Emerging Therapeutics in Cancer: Update on Prostate Cancer, 7th Annual National VA Oncology Symposium, Alexandria, VA, October 2 – 4, 2002.

Nipent® (pentostatin) Experience in the Treatment of CLL. 2005 ASCO Annual Meeting, May 13-17, 2005.

International Vaccine Conference Anti-Idiotype Vaccines Mimicking the CEA and GD-2 Gangliosides, Italy, 2004.

Grand Rounds, University of Pittsburgh School of Pharmacy, Anti-Idiotype Vaccines Mimicking the CEA and GD-2 Gangliosides, Italy, 2004.

ECOG 2005 Fall Meeting, Anti-Idotype Antibody That Mimics the GD2 Gaglioside, Tampa, FL, Nov. 20, 2005.

Reason for Hope – Advances in the Treatment of CLL – "Reason to Hope" Series. UPMC St. Cancer Center – St. Clair, Upper St. Clair, PA, Nov. 30, 2005

3. Other Research Related Activities

<u>Patents</u>

Tri-Gem (1A7)
Patent Number: 5,612,030
Date of Patent: 3/18/97
"Anti-idiotype monoclonal antibody 1A7 and use for the treatment of melanoma and small cell carcinoma"

TriAb (11D10)
Pending Serial Number: 08/766,350
Date Filed: 12/13/96
"Murine monoclonal anti-idiotype antibody 11D10 and methods of use thereof"

CeaVac (3H1)
Pending Serial Number: 08/579940
Date Filed: 12/28/95 (whole antibody)
"Murine monoclonal anti-idiotypic antibody 3H1"

Pending Serial Number: 08/579916
Date Filed: 12/28/95

Member, TVC Advisory Board 2006-present

Member, Spectrum Advisory Board 2006-present

Faculty, Berlex Speakers Bureau 2006-present

Faculty, Genentech Speakers Bureau 2006-present

Faculty, CBCE Speakers Bureau 2003-present

Esai Pharmaceutical Advisory Board	2003-present
Scientific Advisor, Automated Cell	2003-present
Supergen Scientific Advisory Board	2003-present
Editorial Board, Journal of Clinical Immunology	1993-present
Member, Scientific Advisory Board, National Surgical Adjuvant Breast and Bowel Project	1996-present
Editorial Board, Cancer Biotherapy & Radiopharmaceuticals	2005-present
Editor, Journal of Experimental Biology and Medicine, Translational Research Section	2005-present
Co-Editor, Expert Opinion on Biological Therapy, Future Perspectives Section	2006-present
Associate Editor, Clinical Cancer Research	1998-present
Editorial Board, Journal of Immunotherapy	1982-1996
Clinical Sciences Study Section (Subcommittee 4) Division of Research Grants	1986-1990
Associate Editor, Cancer Research	1987-2003
Associate Editor, Antibodies, Immunoconjugates and Radiopharmaceuticals	1987-1990
Editorial Board, Contemporary Oncology	1990-1994
Grants Review Committee for the American Cancer Society	1993-1996
Board of Directors, The Society for Biological Therapy	1995-1997
Editorial Board, Journal of Biotherapy	1995-2000
Board of Advisors, NeoRx Corporation,	1985-1988

Seattle, WA

P01, Member, Memorial Sloan Kettering Cancer Center, NCI Site Visit committee, New York, NY	May 1, 1985
P01, Member, NCI Special Review Committee	October 1, 1985
P01, Member, Stanford Medical Center, NCI Site Visit Committee, Stanford, CA	October 1, 1985
National Research Service Award Advisory Committee, National Institute of Health	1985-1989
P01, University of California at Davis, Sacramento, CA, Member, NCI Site Visit Committee	March 1, 1988
P01, Member, Immunomedics, Site Visit Committee, Morris Plains, NJ	December 5, 1990
P01, Stanford Medical Center, Member, NCI Site Visit Committee, Stanford, CA	November 11, 1991
P30, Member, University of Nebraska Cancer Center, NCI Site Visit Committee, Omaha, NE	February 10, 1992
P50, Member, NCI Special Study Section for AIDS Center Grants	July 13, 1993
Board of Advisors, Inex Pharmaceuitcals Corp. Vancourver, B.C.	1994-1995
Health & Environment Laboratories, Eastman Kodak Company, Rochester, NY	1994-1996
PO1, University of California at Davis, Sacramento, CA, NCI Site Visit Committee Chairman	April 13, 1994
2P01CA59326-03, Gene Therapy for Cancer, University of California at Los Angeles, Member, NCI Site Visit Committee	June 19, 1994
Scientific Review Panel, Israel Cancer Research	1995-2001

Fund, New York, NY

2P01CA4499108, Theray of Lymphoma/Leukemia with Monoclonal Antibodies, Fred Hutchinson Cancer Research Center, Member, NCI Site Visit Committee	February 14, 1995
1P30CA6953301, Oregon Cancer Center, Member, NCI Site Visit Committee	June 12, 1995
1P30CA6953301, Oregon Cancer Center, Member NCI Site Visit Committee	August 3, 1995
NSABP Scientific Advisory Board, Operations Center Site Visit, Pittsburgh, PA	February 6, 1996
2P01CA5935005, Memorial-Sloan Kettering Cancer Center, Gene Therapy Program, NCI Site Visit Committee Chairman	July 7, 1996
1P30CA6953301A1 Oregon Cancer Center, Member, NCI Site Visit Committee	October 7, 1996
Director, Scientific Advisory Board, Titan Pharmaceuticals, Inc., South San Francisco, CA	1996-2001
NCI Scientific Review Group-Subcommittee H	1997-2001
NCI Clinical Oncology Study Section	1999-2001
ACOSOG Site Visit, Chicago, IL	Sept. 21, 1999
SuperGen Advisory Board Meeting, Phoenix, AZ	January 15, 2000
SuperGen Advisory Board Meeting, Phoenix, AZ	January 13-16, 2000
NCI GI Intergroup State-of-Science Symposium	February 15, 2000
NCI Clinical Oncology Study Section, Bethesda, MD	March 20, 2000
IDEC Pharmaceuticals Meeting, San Diego CA	May 7-8, 2000
American College of Surgeons/Tritan Pharmaceutical Meeting, Chicago, IL	May 16, 2000

NCI NIH Scientific Review Group-Subcommittee H Site Review, Rochester, MN	June 7-9, 2000
SuperGen, Rubitecan Advisory Board Meeting Maui, HI	June 13-17, 2000
American College of Surgeons Semiannual Meeting, Chicago, IL	June 24, 2000
NCI Clinical Oncology Study Section, Bethesda, MD	July 7-14, 2000
Abbott Pharmaceutical Advisory Panel, Chicago, IL	Dec. 8-9-, 2000
Fulcrum Renal Cell Advisory Board Mtg. New York	Jan. 14-15, 2001
NCI Breast Intergroup Retreat, Washington, DC	Jan. 18-19, 2001
NCI Scientific Review Group-Subcommittee H Teleconference	March 15, 2001
PO1, Member, NCI Scientific Review Group	November 2005
ICRF Scientific Review Group	March 2006

LIST OF CURRENT RESEARCH INTERESTS:

Current grant funded research is a phase III clinical trial using the TriGem anti-idiotype vaccine for melanoma developed in our laboratories. At the UPCI, I am developing new protocols for a variety of hematologic malignancies including Hodgkin's disease, lymphomas, chronic lymphocytic leukemia, multiple myeloma, chronic myelogenous leukemia, acute myelogenous leukemia, and myelodysplastic syndromes. A collaboration with the NCI and Therion Biopharm for a vaccine therapy of CLL has been established. New collaborations with UPCI investigators are ongoing to generate dendritic cell vaccines for B-cell lymphoma and multiple myeloma. We have established a chronic lymphoid malignancies/multiple myeloma clinical center that will allow us to expand these translational research programs. In the area of solid tumors, we are establishing new areas of translational research with UPCI investigators generating a variety of dendritic cell vaccine approaches, and with investigators at the National Cancer Institute and Biotechnology companies using viral vectors for gene therapy.

SERVICE:

1. University and Medical School

1989 - 1990	Search Committee for Chairman, Department of Radiation Oncology, Roswell Park Cancer Institute
1989 - 1990	Search Committee for Chairman, Department of Cytogenetics, Roswell Park Cancer Institute
1999 –2001	Oncology-Hematology Care Executive Committee, University of Cincinnati
1999 –2001	Ohio Cancer Incidence Surveillance System Advisory Board Ohio Department of Health
1999 –2001	Hematology-Oncology Fellow Evaluation Committee, University of Cincinnati
1989 - 1990	Search Committee for Chairman, Department of Pediatrics, Roswell
1990	Head, Search Committee for Infectious Disease Specialist, Roswell Park Cancer Institute
1990 - 1991	Search Committee for Chairman, Department of Microbiology, SUNY
1989 - 1990	Radiation Safety Committee, Roswell Park Cancer Institute
1989 - 1990	Quality Assurance Committee, Roswell Park Cancer Institute
1989 - 1991	American Society of Hematology, Neoplastic Committee
1990	Head, Search Committee for Pulmonologist/Intensivist, Roswell Park
1991	Head, Search Committee for Cardiologist, Roswell Park Cancer Institute Cancer Institute
1990 - 1991	Vice President, Medical Staff, Roswell Park Cancer Institute
1993	Head, Search Committee for Chairman of Radiation Medicine,
1989 - 1991	American Society of Clinical Oncology, Program Committee
1991 - 1992	American Association for Cancer Research, Program Committee
1992 - 1994	Clinical Immunology Society, Program Committee
1992 -1989	Internal Medicine Chairman's Advisory Committee
1994 –1999	Liaison Committee on Medical Education, University of Kentucky
1994 - 1996	Medical Center Clinical Sciences Area Advisory Committee, University of Kentucky
1996 - 1999	General Clinical Research Center Advisory Committee, University of Kentucky
1999 –2001	Chair, Cancer Steering Committee, University Hospital, University of Cincinnati
1999 –2001	Vontz Steering Committee, University of Cincinnati
1999 –2001	Chair, Internal Advisory Committee, Barrett Cancer Center, University of Cincinnati
1999 –2001	Chair, Scientific Review Committee, Barrett Cancer Center, University of Cincinnati
2003-Present	Director of the University of Pittsburgh Cancer Institute, GCRC

2003-Present	Member of the University of Pittsburgh Medical Center GCRC Advisory Board
2005-Present	Co-Chair, PAC3, Pennsylvania Cancer Control Consortium
2003-Present	Co-Leader, Biologic Therapeutics Program, University of Pittsburgh
2003-Present	Co-Leader, Hematologic Malignancy Program, University of Pittsburgh
2003-Present	Director of Clinical Investigations, University of Pittsburgh Cancer Institute

2. Community Activities

1985-1987	Board of Trustees, Michigan Chapter, Leukemia Society of America
1994-1995	American Cancer Society, Board of Directors, Fayette County Unit
1994 - 1996	Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY
1998	Department of Insurance, Commonwealth of Kentucky

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GOLDENBERG

Serial No.: 10/314,330

Filed: December 9, 2002

Title: IMMUNOTHERAPY OF B-CELL MALIGNANCIES USING ANTI-CD22 ANTIBODIES

Group Art Unit: 1643

Examiner: Alana M. Harris

Attorney Docket No.: IMMU:007US4

EFS-WEB

DECLARATION UNDER 37 CFR § 1.132

MAIL STOP AMENDMENT
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

I, John Leonard, being duly warned, declare as follows:

1. I am the Clinical Director at the Cornell Center for Lymphoma and Myeloma at the New York-Presbyterian Hospital. I have an extensive background in the field of immunotherapy for cancer treatment, as evidenced by my Curriculum Vitae, which is attached. I have been a key investigator on clinical trials relating to immunotherapy of various B-cell malignancies, particularly rituximab. For example, I am currently a principal investigator for a phase II trial that is studying rituximab versus lenalidomide versus rituximab + lenalidomide in recurrent follicular Non-Hodgkin Lymphoma (NHL) after relapse from a rituximab-containing combination regimen.

2. I am familiar with the article Maloney *et al.*, *Blood*, 84(8): 2457-2466 (1994). This article relates to results from a Phase I clinical trial to evaluate the safety of anti-CD20 antibody as a single agent therapeutic.

3. Maloney 1994 states, on page 2465, that "extension of these studies to patients with minimal disease, using antibody alone or in combination with conventional therapies, may provide the greatest benefit. The disclosure in Maloney that anti-CD20 may be combined with a

"conventional therapy" would not have suggested to me therapy with a combination of an anti-CD20 antibody and another antibody, such as an anti-CD22 antibody. This is because "conventional therapies" at the time of the Maloney article, circa 1994, were chemotherapies, not antibody therapies.

4. "Conventional" means "conforming to established practice or accepted standards; traditional" (The American Heritage® Dictionary of the English Language: Fourth Edition - 2000). An investigational drug in Phase I clinical trials cannot be considered a conventional therapy, *i.e.*, it does not conform to established practice or accepted standards." By definition, investigational drugs have not been "accepted." Companies can provide investigational drugs to doctors if they are part of a drug trial covered by an FDA-approved protocol, and such drugs are by definition not conventional, since they are not available for use by any doctor on any patient.

5. In 1994 (and later) antibody therapies were not "conventional," and therefore Maloney's comment regarding the addition of "conventional therapies" to his anti-CD20 antibody suggests to a skilled clinician a combination of the anti-CD20 single antibody therapy with a chemotherapy. It would not have suggested therapy with a combination of antibodies. "Conventional therapies," circa 1994 and later, were chemotherapies. The first approved antibody for therapy of any malignancy was the anti-CD20 antibody rituximab that is the subject of Maloney, but it was not approved until 1997, and therefore there was no cancer therapy with any antibody that was a conventional therapy in 1994.

6. Treatment with anti-CD22 antibody was not conventional circa 1994. For example, Goldenberg *et al.*, *J. Clin. Oncol.*, 9: 548-564 (1991) relates to results from a pilot Phase I study involving a small number of patients to see the feasibility of giving this radiolabeled antibody, involving targeting tumor and organs, doses delivered to tumor and normal organs, and any evidence of efficacy in a small number of patients, and does not establish that treatment with anti-CD22 antibody was "conventional." Juweid *et al. Cancer Res.*, 55:5899s-5907s (1995) is a follow-on report of further treatment of patients with low doses and initial results of high dose therapy in a Phase I trial. Here again, these were early investigational studies and do not represent conventional therapy.

7. Current reviews and texts support the fact that antibody therapy using a combination of antibodies to different targets is not conventional. No such combination therapy has been approved, and even combinations of rituximab with conventional chemotherapy for lymphoma have only been approved by the FDA within the past two years. Although some articles began to discuss

the possibility of combination antibody therapies following publication by Immunomedics of their studies of epratuzumab and rituximab in about 2002/2003, none of these indicate that such therapy is "conventional." For example, "What is New in Lymphoma," published in 2004, cites rituximab as an advancement in the treatment of NHL. Cheson, CA *Cancer J Clin*, Sep-Oct; 54(5):260-72 (2004). Efforts to improve the activity of rituximab are noted, and include increasing the number of weekly infusions, delivering higher doses and increasing dose density. Combinations with CHOP are also mentioned. The Cheson article also references both a Phase II study of a combination of rituximab with epratuzumab and a phase I/II study of the combination of galiximab and rituximab, each of which were reported in 2003, demonstrating that combination antibody therapy was still very much investigational at this later date.

9. Currently I am principal investigator of a phase II study of combination antibody therapy, in this case rituximab plus galiximab (anti-CD80) (currently in press for publication in *Annals of Oncology*). I would not have understood Maloney 1994 to have suggested such a combination antibody therapy based on the statement in the article that "extension of these studies to patients with minimal disease, using antibody alone or in combination with ***conventional therapies***, may provide the greatest benefit." I would have understood Maloney's statement to suggest combinations of the anti-CD20 antibody with chemotherapy, which was "conventional" in 1994. Even single antibody therapy was not conventional in 1994.

****

I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

3/12/07
Date

John P. Leonard, MD

CURRICULUM VITAE

Name: John P. Leonard, M.D.

Date of preparation: January 4, 2007

A. GENERAL INFORMATION

Office address:	Starr Building, Room 340 Division of Hematology/Oncology Weill Medical College of Cornell University 520 East 70th Street New York, NY 10021
Office telephone:	(212) 746-2932
Office fax:	(212) 746-3844
Home address:	4556 Boston Post Road Pelham Manor, NY 10803
Home telephone:	(914) 738-0630
Cell phone:	(917) 696-2168
Beeper:	(212) 746-6700 # 16668
Email:	jpleonar@med.cornell.edu
Citizenship:	USA

Optional information

Birth date:	June 13, 1965
Birth place:	Virginia
Social Security Number:	Upon request
Marital status:	Married
Spouse's name:	Leah Smith Leonard
Children's name and ages:	Madeline (10), Abigail (7), Zachary (5)
Race/Ethnicity:	Caucasian

B. EDUCATIONAL BACKGROUND

Degree	*Institution name, city and state*	*Dates attended*	*Year Awarded*
B.A.	Johns Hopkins University Baltimore, Maryland	1982-1986	1986
M.D.	University of Virginia Charlottesville, Virginia	1986-1990	1990

C. PROFESSIONAL POSITIONS AND EMPLOYMENT

Post-doctoral training including residency/fellowship

Title	*Institution name, city and state*	*Dates*
Intern, Resident, Assistant Chief Resident	Department of Medicine New York Hospital-Cornell Medical Center, New York, New York Memorial Sloan-Kettering Cancer Center, New York, New York	1990-1993
Fellow, Chief Fellow	Division of Hematology-Oncology New York Hospital – Cornell Medical Center, New York, New York	1993-1996

Academic positions (teaching and research)

Title	*Institution name, city and state*	*Dates*
Instructor in Medicine	Division of Hematology-Oncology Department of Medicine Weill Medical College of Cornell University	1995-1996
Chief Resident	Department of Medicine New York Hospital – Cornell Medical Center, New York, New York	1996-1997
Senior Clinical Associate in Medicine	Department of Medicine Weill Medical College of Cornell University, New York, New York	1996-1997
Assistant Professor of Medicine	Division of Hematology-Oncology Department of Medicine Weill Medical College of Cornell University, New York, New York	1997-2004
Associate Professor of Medicine	Division of Hematology-Oncology Department of Medicine Weill Medical College of Cornell University, New York, New York	2004-present

Hospital positions (attending physician)

Title		*Institution name, city and state*	*Dates*
Assistant Physician	Attending	New York Weill Cornell Medical Center New York Presbyterian Hospital New York, New York	1995-2004
Associate Physician	Attending	New York Weill Cornell Medical Center New York Presbyterian Hospital New York, New York	2004-present

Employment (other than positions listed above)

N/A

D. LICENSURE, BOARD CERTIFICATION, MALPRACTICE (if applicable)

Licensure

State	*Number*	*Date of Issue*	*Date of last registration*
New York	186477	1991	2006

DEA number: BL 3656541

Board Certification

Name of specialty	*Board Certificate #*	*Date of Certification*
Internal Medicine	148759	1993
Hematology	148759	1996, 2006
Medical Oncology	148759	1997, pending

Malpractice insurance

Do you have Malpractice insurance? Yes

Name of Provider: MCIC Vermont (through Weill Cornell)

Premiums paid by: *(self/ group/ institution (give name of group/institution)*
Weill Medical College of Cornell University

E. PROFESSIONAL MEMBERSHIPS (*medical and scientific societies*)

Member/officer	*Name of Organization*	*Dates held*
Member	American College of Physicians	1993
Member	American Federation for Medical Research	1993
Member	American Society of Hematology	1994
Member	American Society of Clinical Oncology	1997
Member	Cancer and Leukemia Group B	2000
Member	New York Cancer Society	2001

F. HONORS AND AWARDS

Name of award	*Date awarded*
Raven Society, University of Virginia	1989
Medical Alumni Association Outstanding Medical Student Award, University of Virginia School of Medicine	1990
Medical Housestaff Program Director's Award, Department of Medicine, New York Hospital – Cornell Medical Center	1993
Chief Medical Resident, Department of Medicine, New York Hospital – Cornell Medical Center	1996
Mentored Patient-Oriented Research Career Development Award (K23), National Institutes of Health	2001
First Prize, Department of Medicine Investigator Award, Weill Medical College of Cornell University	2005

G. INSTITUTIONAL/HOSPITAL AFFILIATION

Primary Hospital Affiliation:	New York Weill Cornell Medical Center New York Presbyterian Hospital New York, New York
Other Hospital Affiliations:	N/A
Other Institutional Affiliations:	N/A

H. EMPLOYMENT STATUS

Name of Employer(s): Weill Medical College of Cornell University

Employment Status: Full-time salaried by Weill Cornell

I. CURRENT AND PAST INSTITUTIONAL RESPONSIBILITIES AND PERCENT EFFORT

Teaching	Dates
Chief Medical Resident, Department of Medicine	1996-1997
Ward teaching attending, 2-4 months/year (3 hrs/day teaching rounds with students, fellows and housestaff while attending on service)	1997-present
Laboratory session instructor for medical students including Basis of Disease course (Lymphoma laboratory, 2 hours/year)	1997-present
Hematology/Oncology lectures to medical students and housestaff (4 informal lectures/year to students/housestaff on "lymphoma basics", generally 1 formal lecture/year on "Non-Hodgkin's Lymphoma)	1997-present
Hematology/Oncology lectures to fellows (1-2/year on "Indolent lymphoma" and "Aggressive lymphoma")	1997-present
Morning report teaching attending, Department of Medicine (1-2 weeks/year)	1997-present
Hematology/Oncology Lymphoma Clinic attending (supervising fellows and rotating residents/students – generally 10-15 hours/week)	2000-present
Research mentor for trainees	2001-present

Fellows

Richard Furman, M.D. – currently Assistant Professor of Medicine, Weill Medical College of Cornell University

Alan Dosik, M.D. – currently Attending Physician, New York Methodist Hospital

Abby Siegel, M.D. – currently Assistant Professor of Medicine, Columbia University College of Physicians and Surgeons

Jia Ruan, M.D. – currently Assistant Professor of Medicine, Weill Medical College of Cornell University

Biree Andemariam, M.D. – current 3rd year fellow

Medical Residents
Geoffrey Ku, M.D.
Jody Mones, M.D.

Medical Students
Elena Schoenberger
Sarah Rutherford

Course Director (medical students), Malignant Hematology (4th year elective rotation, approximately 5 students/year)	2003-present
Director, Hematology-Oncology Fellowship Program (3 fellows/year)	2003-2005

Clinical Care	Dates
Ward attending (2-4 months/year) and Lymphoma clinic (outpatient) attending (2 days/week)	1997-present

Administrative duties	Dates
Housestaff Committee, Department of Medicine, New York Hospital – Cornell Medical Center	1990-1993, 1996-1997
Intern Selection Committee, Department of Medicine, New York Weill Cornell Medical Center	1993-present
Cardiac Arrest Committee, New York Hospital – Cornell Medical Center	1996-1997
Task Force on Patient Restraints, New York Hospital – Cornell Medical Center	1996-1997
Quality Assurance Committee, Department of Medicine, New York Weill Cornell Medical Center	1996-1997, 1998-2005
Oncology Cluster Committee (Medical Director), New York Weill Cornell Medical Center	1997-2001
Director, Inpatient Oncology Unit, New York Weill Cornell Medical Center	1997-2003
Medical Director, Oncology Services, New York Weill Cornell Medical Center	1999-2003
Clinical Director, New York-Cornell Center for Lymphoma and Myeloma	1998-present

Hematology/Oncology Subcommittee, Formulary and Therapeutics Committee, New York Presbyterian Hospital	2000-2003
Oncology Operations Council/Bicampus Cancer Council, New York Weill Cornell Medical Center	2001-present
Clinical Protocol Review Committee, Division of Hematology-Oncology, Weill Medical College of Cornell University (Chair, 2005-present)	2001-present
Radiation Safety Committee, New York Weill Cornell Medical Center	2002-present
Director, Hematology-Oncology Fellowship Program, New York Weill Cornell Medical Center (responsible for training, clinical and research activities of 3 fellows/year)	2003-2005
Associate Director, Clinical Research Program, Division of Hematology/Oncology	2004-2005
Director, Clinical Research Program, Division of Hematology/Oncology (supervise clinical trials office, responsible for management of 35+ staff and average of 80+ ongoing trials and 400+ patient accruals/year, funded through National Cancer Institute, foundation, industry and institutional support)	2005-present
Director of Clinical Research Development, Institute for Clinical Research, Weill Medical College of Cornell University (collaborate with ICR leadership to focus on development of new programs to enhance the quality and extent of clinical and translational research programs at Weill Cornell)	2006-present

<u>Research</u>	<u>Dates</u>
Regulation of hematopoiesis, Division of Experimental Hematology, Johns Hopkins Oncology Center (Johns Hopkins School of Medicine), Saul Sharkis, PhD	1984-1990

Gene therapy in hematopoietic cells, Laboratory of Developmental Hematopoiesis (Sloan Kettering Institute), Malcolm A.S. Moore, D Phil	1994-1996
Immunotherapy, radioimmunotherapy, chemotherapy and other novel therapeutic approaches for non-Hodgkin's lymphoma and Hodgkin's disease	1997-present
New imaging modalities for lymphoma	1997-present
Member, Lymphoma Core Committee, Cancer and Leukemia Group B, National Cancer Institute	1998-present
Leader, Non-Hodgkin's Lymphoma Working Group, Lymphoma Core Committee, Cancer and Leukemia Group B, National Cancer Institute (work with other committee leadership to establish the portfolio of clinical and translational research in lymphoma in CALGB, a cooperative group of the NCI with 25+ academic main member institutions nationally)	2003-present
Principal Investigator (Weill Medical College of Cornell University), Cancer and Leukemia Group B (Main Member Institution), National Cancer Institute	2006-present
Principal Investigator (Weill Medical College of Cornell University site) and Executive Committee, New York Cancer Consortium, National Cancer Institute (one of 8 phase II cancer consortia of the NCI nationally)	2006-present

Check if activity involves WMC

Current percent effort	*%*	*students*	*researchers*
Teaching	5%	x	x
Clinical Care	45%	x	x
Administration	20%	x	x
Research	30%	x	x
Total	100%		

J. RESEARCH SUPPORT (past and present)

NIH, peer-reviewed, investigator-initiated:

K23 - RR16814, Mentored Patient-Oriented Research Career Development Award, “Novel monoclonal antibody therapies for lymphoma”

Source	*Amount*	*Date*	*Name of Principal Investigator*
National Institutes of Health, National Center for Research Resources	$540,000	9/1/01-8/31/06	John P. Leonard

Individual's role in project including percent effort
Principal investigator (75% effort)

UO1 – HL72196, “Novel therapies in hemostasis and transfusion medicine”

Source	*Amount*	*Date*	*Name of Principal Investigator*
National Institutes of Health – National Heart, Lung, Blood Institute		2002-2007	James B. Bussel

Individual's role in project including percent effort
Co-investigator (5% effort)

U10 –CA31946, “Cancer and Leukemia Group B”(subcontract), CALGB Foundation, and Clinical Trials Support Unit (CTSU)

Source	*Amount*	*Date*	*Name of Principal Investigator*
National Institutes of Health, National Cancer Institute	$123,850 (2005-6)	2005-2009	Richard L. Schilsky

Individual's role in project including percent effort
Principal investigator, Main Member Institution (New York Weill Cornell)

N01- CM-17103 - NCI Phase II contract, “Early therapeutics development with phase II emphasis, New York Cancer Consortium”(subcontract)

Source	*Amount*	*Date*	*Name of Principal Investigator*
National Institutes of Health, National Cancer Institute	$338,300 (2005-6)	2005-2010	Joseph Sparano

Individual's role in project including percent effort
Principal investigator (New York Weill Cornell Site) and Member, Executive Committee

R21 – CA126060 , Quick-Trials for Novel Cancer Therapies, “CD74-directed immunotherapy for B cell malignancies” (Anticipated award – Priority score 128, 2.6 percentile with current payline 8%)

Source	*Amount*	*Date*	*Name of Principal Investigator*
National Institutes of Health, National Cancer Institute	$521,093 direct costs	2007-2009	John P. Leonard

Individual's role in project including percent effort
Principal investigator (25% effort)

Foundation or institutional, peer-reviewed, investigator-initiated

Pilot Grant in Aging Research, “Monoclonal antibody-based immunotherapy in older patients with non-Hodgkin's lymphoma”

Source	*Amount*	*Date*	*Name of Principal Investigator*
Cornell Center for Aging Research and Clinical	$20,000	2002-2003	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

Research grant, “Clinical, pathologic and molecular correlative studies in lymphoma”

Source	*Amount*	*Date*	*Name of Principal Investigator*
Dorothy Rodbell Cohen Foundation	$50,000	2003-2004	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

Mantle cell lymphoma research grant (“R01 type”), “Angiogenesis and anti-angiogenic therapy in mantle cell lymphoma”

Source	*Amount*	*Date*	*Name of Principal Investigator*
Lymphoma Research Foundation	$950,000	2003-2007	John P. Leonard

Individual's role in project including percent effort
Principal investigator (10% effort)

Research grant, “Monoclonal antibody-based therapies for B cell malignancies”

Source	*Amount*	*Date*	*Name of Principal Investigator*
The Lymphoma Foundation	$30,000	2005-2007	John P. Leonard

Individual's role in project including percent effort
Principal investigator (10% effort)

Pending foundation or institutional, peer-reviewed, investigator-initiated

CLL/SLL research grant ("R01 type"), "CD74-directed therapy of CLL/SLL"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Lymphoma Research Foundation	$750,000 direct costs	Pending; 2007-2010	John P. Leonard

Individual's role in project including percent effort
Principal investigator (5% effort)

Mantle cell lymphoma research grant ("R01 type"), "Annexin 2 in Mantle Cell Lymphoma Angiogenesis"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Lymphoma Research Foundation	$663,177 direct costs	Pending; 2007-2010	Katherine A. Hajjar

Individual's role in project including percent effort
Co-investigator (5% effort)

Industry-sponsored, investigator-initiated clinical trials

"Multicenter, pivotal phase III study of Iodine-131 anti-B1 antibody (murine) radioimmunotherapy for chemotherapy-refractory low-grade B-cell lymphomas and low-grade B-cell lymphomas that have transformed to higher grade histologies"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$5,150	1998-ongoing	Stanley J. Goldsmith

Individual's role in project including percent effort
Co-investigator (<5% effort)

"A randomized study of Iodine-131 anti-B1 antibody versus anti-B1 antibody in chemotherapy-relapsed/refractory low-grade of transformed low-grade non-Hodgkin's lymphoma (NHL)"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$336,468	1999-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Retreatment study of patients with non-Hodgkin's lymphoma who have previously responded to Iodine-131 antibody"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$8,000	1998-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Fludarabine monophosphate followed by Iodine-131 anti-B1 antibody for untreated low-grade and follicular non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$165,331	1998-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase I/II clinical trial of immunotherapy with Mab hLL2 in patients with recurrent non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Immunomedics, Inc	$369,055	1998-ongong	John P. Leonard
Amgen, Inc		1998-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Expanded access study of Iodine-131 anti-B1 antibody for relapsed/refractory low-grade and transformed low-grade NHL"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$166,125	1999-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase I, dose-escalation study of Iodine-131 anti-B1 antibody for patients with previously treated non-Hodgkin's lymphoma with more than 25% bone marrow involvement"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$53,000	1999-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Data management support grant"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation		1999-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase I trial of humanized 1D10 monoclonal antibody (Hu1D10) in patients with relapsed non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Protein Design Labs		1999-2000	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase II multicenter study of Iodine-131 anti-B1 antibody consolidation for patients with diffuse large B-cell non-Hodgkin's lymphoma following first-line CHOP"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$23,500	2000-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase II multicenter study of CVP followed by Iodine-131 anti-B1 antibody for patients with untreated low-grade non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Corixa Corporation	$81,057	2000-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II trial of immunotherapy with humanized LL2 (epratuzumab) in combination with rituximab in patients with refractory or recurrent non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Immunomedics, Inc., and Amgen, Inc.	$129.131	2000-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase III trial evaluating the safety and efficacy of specific immunotherapy, recombinant idiotype conjugated to KLH with GM-CSF, in patients with follicular non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Genitope Corporation	$229,406	2000-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II trial to evaluate the rate of immune response using idiotype immunotherapies produced by molecular biological means for treatment of aggressive B-cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Genitope Corporation	$49,850	2000-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase III clinical trial of immunotherapy with humanized LL2 IgG (AMG 412) in subjects with low-grade, follicular, B-cell non-Hodgkin's lymphoma refractory to rituximab"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Amgen, Inc.	$44,197	2000-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Randomized study of fludarabine and cyclophosphamide with or without Bcl-2 antisense oligonucleotide in patients with relapsed or refractory chronic lymphocytic leukemia"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Genta, Inc.	$12,500	2002-2005	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A randomized phase III multicenter controlled clinical trial to evaluate the efficacy and safety of IDEC-Y2B8 radioimmunotherapy compared to rituximab immunotherapy of relapsed or refractory low-grade or follicular B-cell non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Idec Pharmaceuticals		1999-2001	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II clinical trial testing AMG 412 (anti-CD22, epratuzumab) in combination with rituximab in rituximab-naïve patients with refractory or recurrent low-grade CD20+ B-cell non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Amgen, Inc.	$30,400	2002-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase II study of FavId (tumor-specific idiotype-KLH) and soluble GM-CSF immunotherapy in patients with stable or progressive low-grade follicular B-cell lymphomas"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Favrille, Inc.	$20,840	2002-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase II open label, randomized, dose and schedule finding, clinical trial of immunotherapy with AMG 412 (epratuzumab, anti-CD22) in subjects with diffuse large B-cell non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Amgen, Inc.	$9,900	2002-2003	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

A phase I/II trial of anti-CD80 monoclonal antibody (IDEC-114) therapy for patients with relapsed or refractory follicular lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Idec Pharmaceuticals	$34,000	2002-2006	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Multicenter, phase I, open label, two arm, non-randomized, dose-escalation study of the safety and tolerability of CpG 7909 in patients receiving rituximab for relapsed or refractory B-cell non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Coley Pharmaceuticals	$82,045	2002-2006	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Multicenter study of Bcl-2 antisense alone or in combination with rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) in patients with newly diagnosed, refractory or relapsed mantle cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Genta, Inc.	$148,000	2002-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Open label phase II/III study of rituximab in combination with recombinant human IL-2 for relapsed low-grade or follicular non-Hodgkin's lymphoma in subjects who have previously failed rituximab"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Chiron, Inc.	$30,720	2002-2005	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase I/II multi-dose study of SGN-30 (anti-CD30) in patients with refractory or recurrent CD30+ hematologic malignancies"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Seattle Genetics, Inc.	$36,042	2002-2006	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase II study of intravenous T900607-sodium in subjects with previously treated non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Tularik, Inc.	$8,400	2002-2003	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase I/II trial of IDEC-114 (anti-CD80 monoclonal antibody) in combination with rituximab for patients with relapsed or refractory follicular lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Idec Pharmaceuticals	$116,241	2002-2006	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II multicenter study of gallium nitrate in patients with refractory non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Genta, Inc.	$46,550	2002-2005	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II study to evaluate safety and efficacy of specific immunotherapy, recombinant idiotype conjugated to KLH and GM-CSF following the anti-CD20 antibody rituximab in previously treated patients with follicular non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Genitope, Inc.	$100,444	2003-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II study of Yttrium-90 labeled ibritumomab tiuxetan and rituximab in relapsed diffuse large B-cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Idec Pharmaceuticals	$49,500	2003-2006	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase I/II trial of bortezomib (PS-341) and CHOP-rituximab in previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Millenium Pharmaceuticals	$261,000	2003-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase 2 study of Velcade (bortezomib) in subjects with relapsed or refractory mantle cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Millenium Pharmaceuticals	$36,759	2003-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase II multi-dose study of SGN-30 (anti-CD30 mAb) in patients with refractory or recurrent Hodgkin's disease or anaplastic large cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Seattle Genetics	$54,865	2003-2006	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"A phase I study of immunotherapy with hA20 administered once weekly for 4 consecutive weeks in patients with low-grade, follicular non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Immunomedics	$68,105	2004-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Phase II trial of anti-angiogenic therapy with RT-PEP-C in patients with relapsed mantle cell lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Lymphoma Research Foundation		2004-ongoing	John P. Leonard

Individual's role in project including percent effort
Co-principal investigator (<5% effort)

"Intravenous administration of SB-743921 on days 1 and 15 of a 28-day cycle in non-Hodgkin's lymphoma"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Cytokinetics, Inc		2006-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"Single-agent AT-101 in relapsed or refractory B-cell malignancies"

Source	*Amount*	*Date*	*Name of Principal Investigator*
Ascenta, Inc.	$4,958	2006-ongoing	John P. Leonard

Individual's role in project including percent effort
Principal investigator (<5% effort)

"CALGB 50401 – A randomized phase II trial of rituximab vs. lenalidomide (Revlimid™, CC-5013) (IND#73034) vs. rituximab _ lenalidomide in recurrent follicular non-Hodgkin's lymphoma (NHL) after relapse from a rituximab-containing regimen"

Source	*Amount*	*Date*	*Name of Principal Investigator*
CALGB		2006-ongoing	John P. Leonard

Individual's role in project including percent effort
Overall National Principal investigator (<5% effort)

K. EXTRAMURAL PROFESSIONAL RESPONSIBILITIES

Reviewer for *Annals of Internal Medicine*	1998-
Reviewer for *Cancer*	1998-
Reviewer for *Cancer Investigation*	1998-
Member, Lymphoma Core Committee, Cancer and Leukemia Group B (CALGB), National Cancer Institute	2000-
Member, Medical Affiliates Board, Lymphoma Research Foundation	2001-
Reviewer, *Leukemia and Lymphoma*	2002-
Reviewer, *Blood*	2002-
Member, Editorial Board, *Clinical Lymphoma*	2002-
Abstract Reviewer, American Society of Hematology Annual Meeting	2002
Co-chairman, Lymphoma/Myeloma 2002 (international meeting), New York, NY	2002
Member, Public Policy Committee, Lymphoma Research Foundation	2003 -

Member, Editorial Board, *Journal of Clinical Oncology*	2003-2005
Coordinating Abstract Reviewer, American Society of Hematology Annual Meeting	2003
Leader, Non-Hodgkin's Lymphoma Working Group, Lymphoma Core Committee, Cancer and Leukemia Group B (CALGB), National Cancer Institute	2003
Member, Scientific Advisory Board, Lymphoma Research Foundation (International foundation dedicated to improvement in treatment and support for patients with lymphoma)	2003
Co-chairman, Lymphoma/Myeloma 2004 (international meeting – 600 participants), New York, NY	2004
Reviewer, Translational Research program, Leukemia and Lymphoma Society (International foundation dedicated to improvement in treatment and support for patients with leukemia, lymphoma and myeloma)	2004-
Member, expert panel of advisors for strategic planning, Leukemia and Lymphoma Society	2005
Member, Executive Committee, Mantle Cell Consortium, Lymphoma Research Foundation	2005-
External Reviewer, Cancer Therapy and Evaluation Program, National Cancer Institute	2005-
Member, Government Affairs Committee, American Society of Hematology	2005-
Member, Board of Directors, Lymphoma Foundation	2005-
Abstract Reviewer, American Society of Hematology	2006

Co-chairman, Lymphoma/Myeloma 2006 (international meeting – 800 participants), New York, NY	2006
Member, Board of Directors, National Coalition for Cancer Research	2007-
Member, Editorial Board, *Blood*	2007-

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49. **Leonard JP** and Goldenberg DM. Preclinical and clinical evaluation of epratuzumab (anti-CD22 IgG) in B cell malignancies, Oncogene, in press.

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51. **Leonard JP**, Friedberg JW, Younes A, Fisher D, Gordon LI, Moore J, Czuczman M, Miller T, Stiff P, Cheson BD, Forero-Torres A, Chieffo N, McKinney B, Finucane D, Molina A. A phase I/II study of galiximab (an anti-CD80 monoclonal antibody) in combination with rituximab for relapsed or refractory, follicular lymphoma, Annals of Oncology, accepted pending minor revision.

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54. Weng W and **Leonard JP**. Personalized active immunotherapy for non-Hodgkin's lymphoma: Mechanisms of efficacy, submitted.

55. Bartlett N, Younes A, Carabasi M, Forero A, Rosenblatt J, **Leonard JP**, Bernstein S, Bociek RG, Barton J. A phase I multi-dose study of SGN-30 immunotherapy in patients with refractory or recurrent CD30+ hematologic malignancies, submitted.

56. **Leonard JP**, Emmanouilides C, Gregory SA, Weisdorf D, Andrey J, Hainsworth J, Link B, Sparano J, Tsai D, Horning SJ, Kurman M, Krieg AM and Weiner GJ. A phase I trial of TLR9 agonist PF-3512676 (CpG 7909) in Relapsed or refractory non-Hodgkin's lymphoma patients with or after Rituximab, submitted.

Editorials, Books, Book Chapters

1. **Leonard JP** and Coleman M. Primary non-Hodgkin's lymphoma of bone. Cancer Invest, 1998;16:616-617.

2. **Leonard JP**. Epratuzumab (hLL2, anti-CD22) immunotherapy of non-Hodgkin's lymphoma. Hematologica, 2001, 86(S1):80-83.

3. **Leonard JP**. Extended duration neutrophil support for patients undergoing myelosuppressive chemotherapy. Curr Hematol Rep 2002 1(2), 93-94.

4. **Leonard JP** and Silverstein RL. "Corticosteroids in hematologic diseases". Chapter 28 in Principles of Corticosteroid Therapy, eds. Lin AN and Paget S. Chapman and Hall, 2002, New York, NY.

5. **Leonard JP**, Petryk M, Grossbard ML. "Monoclonal antibody therapy of lymphoma". Chapter 18 in American Cancer Society Atlas of Clinical Oncology, Malignant Lymphomas, ed. Grossbard ML, 2002. BC Decker.

6. **Leonard JP** and Coleman M. "Non-Hodgkin's Lymphoma", Conn's Current Therapy, ed. Rakel RE, 2002, Lippincott Williams and Wilkins, Philadelphia.514.

7. **Leonard JP**. "The 'FLIPI' is no 'FLOPI' ". Blood 2004, 104: 1233-1234.

8. **Leonard JP** and Coleman M, eds, Hodgkin's and Non-Hodgkin's Lymphoma, 2006, Springer

9. Siegel AB and **Leonard JP**. "Therapy for older patients with diffuse large cell lymphoma: Targeting the treatment to the patient". Leukemia and Lymphoma, in press.

10. Furman RR, **Leonard JP**, Decter J, Coleman, M. "Monoclonal antibodies in lymphoma", Gewirtz M, ed, Apoptosis and Senescence in Oncology, in press.

Selected Abstracts: (First-authored, oral abstract presentations at selected meetings)

1. **Leonard, JP,** Coleman M, Chadburn A, Matthews JC, Bayer R., Schuster, MW, Feldman EJ, Juweid M, Schuster SJ, Wegener WA, Goldenberg DM. Epratuzumab (HLL2, anti-CD22 humanized monoclonal antibody) is an active and well-tolerated therapy for refractory/relapsed diffuse large B-cell non-Hodgkin's lymphoma (NHL). Blood 96: 578a, 2000. Oral presentation at 2000 meeting of the American Society of Hematology.

2. **Leonard JP**, Coleman M, Kostakoglu L, Chadburn A, Cesarman E, Hack S, Kroll SM, Tidmarsh G, Vallabhajosula S, Goldsmith SJ. Triple modality therapy for follicular low-grade lymphoma: Initial treatment with fludarabine followed by Bexxar^{TM} (tositumomab and Iodine I 131 tositumomab). Blood 98:3505, 2001. Oral presentation at 2001 meeting of the American Society of Hematology.

3. **Leonard JP**, Coleman M, Matthews JC, Fiore JM, Dosik A, Kapushoc H, Kin E, Cesano A, Wegener WA, Goldenberg DM. Combination monoclonal antibody therapy for lymphoma: Treatment with epratuzumab (anti-CD22) and rituximab (Anti-CD20) is well tolerated. Blood 98:3506, 2001. Oral presentation at 2001 meeting of the American Society of Hematology.

4. **Leonard JP**, Coleman M, Vose J, Hainsworth JD, Piro L, Saleh M, Bernstein S, Forero-Torres A, Frankel SR, Itri LM. Phase II study of oblimersen sodium (G3139, Bcl-2 antisense) alone and with R-CHOP in mantle cell lymphoma (MCL). Proceedings of the American Society of Clinical Oncology 22:566, 2003. Oral presentation at 2003 meeting of the American Society of Clinical Oncology.

5. **Leonard JP**, Vose J, Timmerman J, Levy R, Coleman M, King S, Ingolia D, Denney D. Recombinant idiotype-KLH vaccination (MyVax) following CHOP chemotherapy in mantle cell lymphoma. Blood 2003. Oral presentation at 2003 meeting of the American Society of Hematology.

6. **Leonard JP**, Hainsworth J, Bernstein S, Forero-Torres A, Vose J, Piro L, Saleh M, Coleman M, Frankel S, Smith S, Itri L. Genasense (Oblimersen sodium, G3139) is active and well tolerated both alone and with R-CHOP in mantle cell lymphoma (MCL). Blood 2003. Oral presentation at 2003 meeting of the American Society of Hematology.

7. **Leonard JP**, Zelenetz AD, Vose JM, Kaminski MS. Tositumomab and iodine I 131 tositumomab (the Bexxar therapeutic regimen) produces higher response rates and longer response durations than prior chemotherapy. Blood 2004. Oral presentation at 2004 meeting of the American Society of Hematology.

8. **Leonard JP**, Coleman MS, Link BK, et al. FLIPI predicts outcome in 65 patients with previously untreated indolent NHL who received bexxar in combination with chemotherapy. Ann Oncol 2005. Oral presentation at 9th International Conference on Malignant Lymphoma, Lugano Switzerland 2005.

9. **Leonard JP**, Friedberg J, Younes A, et al Results from a phase I/II study of galiximab (anti-CD80) in combination with rituximab (anti-CD20) for a relapsed or refractory, follicular NHL. Ann Oncol 2005. Oral presentation at 9th International Conference on Malignant Lymphoma, Lugano Switzerland 2005.

10. **Leonard JP**, Furman RR, Cheung YK, et al. Phase I/II trial of bortezomib + CHOP-rituximab in diffuse large B cell (DLBCL) and mantle cell lymphoma (MCL): Phase I results. Blood 2005. Oral presentation at 2005 meeting of the American Society of Hematology.

Presentations/Invited Lectures: (Selected regional, national and international meetings)

2000 "Novel immunotherapeutic strategies for low- and intermediate-grade non-Hodgkin's lymphoma", Novel Cytokine and Therapeutic Strategies, Super Friday Symposium, American Society of Hematology annual meeting, San Francisco, CA

2000 "Iodine I-131 tositumomab as upfront therapy for NHL", Managing Non-Hodgkin's Lymphoma in the New Millenium, Super Friday Symposium, American Society of Hematology annual meeting, San Francisco, CA

2001 "Iodine-131 tositumomab and chemotherapy as NHL therapy", Winter Oncology Conference, Whistler, British Columbia, Canada

2001 "Targeting CD22 in patients with relapsed NHL", First International Congress on Monoclonal Antibodies in Cancer, Banff, Alberta Canada

2001 "What is the advantage of the new monoclonal anti-CD22 antibody?", Lymphoma…the next questions symposium, Washington, D.C.

2001 "CD22-directed immunotherapy of lymphoma", Society for Biological Therapy 16th Annual Meeting, Bethesda, MD

2001 "Indolent lymphomas – Epratuzumab", New Drugs in Hematologic Malignancies symposium, Bologna, Italy

2001 "Alternative targets for immunotherapy – targeting CD22 with monoclonal antibodies for the treatment of non-Hodgkin's lymphoma", Super Friday Symposium, American Society of Hematology annual meeting, Orlando, FL

2001 "Monoclonal antibodies as single agents for non-Hodgkin's lymphoma", Education Session, American Society of Hematology annual meeting, Orlando, FL

2002 "Epratuzumab and other novel monoclonal antibodies for Lymphoma", Winter Oncology Conference, Whistler, British Columbia, Canada

2002 "Monoclonal antibodies for non-Hodgkin's lymphoma", invited speaker, AIDS Malignancy Consortium, National Cancer Institute, National Institutes of Health, Bethesda MD

2002	“Epratuzumab (anti-CD22) immunotherapy for NHL”, Second International Congress on Monoclonal Antibodies in Cancer, Banff, Alberta, Canada
2002	“Iodine-131 labeled anti-B1 antibody for NHL”, Second International Congress on Monoclonal Antibodies in Cancer, Banff, Alberta, Canada
2002	“Epratuzumab (anti-CD22) therapy for B cell malignancies”, Conference on “Innovative therapies for lymphoid malignancies”, Palermo, Italy
2002	“Radioimmunotherapy of non-Hodgkin’s lymphoma”, Chemotherapy Foundation Symposium XXI, New York, NY
2002	“Idiotype vaccination for NHL”, Chemotherapy Foundation Symposium XXI, New York, NY
2002	“Targeting CD22 as immunotherapy for Non-Hodgkin’s Lymphoma”, Super Friday Symposium, American Society of Hematology Annual Meeting, Philadelphia PA
2003	“New developments in immunotherapy for non-Hodgkin’s lymphoma”, 9th Aichi Cancer Center International Symposium, Nagoya, Japan
2003	“Radioimmunotherapy of lymphoma”, Society of Nuclear Medicine Mid-Winter Educational Symposium, Hollywood, FL
2003	“Epratuzumab and other novel monoclonal antibodies for NHL”, Winter Oncology Conference, Whistler, British Columbia, Canada
2003	“Epratuzumab in the treatment of B-NHL”, First International Symposium on Childhood and Adolescent Non-Hodgkin’s Lymphoma, New York, NY
2003	“New developments in radioimmunotherapy for lymphoma”, Pediatric Grand Rounds, Memorial Sloan-Kettering Cancer Center, New York NY
2003	“Monoclonal antibodies”, invited speaker, State of the Science Symposium on Acute Lymphoblastic Leukemia, National Cancer Institute, Bethesda, MD
2003	“Vaccine and other novel therapies for NHL”, Lymphoma…the next questions symposium, San Juan, Puerto Rico

2003	"Anti-CD22 monoclonal antibody – where will it fit in the management of lymphoma?", Lymphoma…the next questions symposium, San Juan, Puerto Rico
2003	"New developments in immunotherapy for lymphoma", Hematologic Oncology Grand Rounds, Memorial Sloan-Kettering Cancer Center, New York, NY
2003	"Epratuzumab", invited speaker, Pan-Pacific Lymphoma Conference, Kona HI
2003	"Future developments in antibody therapy of lymphoma", invited speaker, Conference on Post-Transplant Lymphoproliferative Disorders, National Institutes of Health, Bethesda MD
2003	"Initial therapy of NHL", Emerging therapies in hematologic malignancies symposium, Super Friday Symposium, American Society of Hematology annual meeting, San Diego CA
2004	Invited discussant, "Radioimmunotherapy. Where should we use it? Why don't we use it?". Nuclear Medicine Section, New York Academy of Medicine, New York NY
2004	"Epratuzumab", Winter Oncology Conference, Whistler, British Columbia, Canada
2004	"Idiotype vaccines for NHL", Winter Oncology Conference, Whistler, British Columbia, Canada
2004	"New developments in immunotherapy of B cell malignancies", invited speaker, Children's Oncology Group meeting, Washington, D.C.
2004	"New developments in radioimmunotherapy of lymphoma", Hematology/Oncology Grand Rounds, SUNY – Stony Brook, NY
2004	"New developments in immunotherapy of B cell malignancies", Leukemia Grand Rounds, MD Anderson Cancer Center, Houston TX
2004	"Novel approaches for the treatment of NHL", Lymphoma/Myeloma Grand Rounds, MD Anderson Cancer Center, Houston TX
2004	"New developments in immunotherapy of B cell malignancies", Lymphoma Conference, Johns Hopkins Oncology Center

2004	Invited discussant, “Rituximab in initial therapy for NHL”, Hematologic malignancies oral presentation session, American Society of Clinical Oncology annual meeting, New Orleans LA
2004	“New developments in radioimmunotherapy for lymphoma”, Hematology/Oncology Grand Rounds, Moffitt Cancer Center, Tampa FL
2004	“CD22-directed immunotherapy for lymphoma”, Fourth International Congress on Monoclonal Antibodies in Cancer, Colorado Springs, CO
2004	“Epratuzumab”, “Tenth anniversary of the REAL Classification – Open Issues” conference, invited speaker, Bologna Italy
2004	“Novel antibody combinations for lymphoma”, Lymphoma/Myeloma 2004, NY NY
2004	“New developments in radioimmunotherapy for lymphoma”, Medical Grand Rounds, Roswell Park Cancer Institute, Buffalo NY
2004	“Initial therapy of NHL”, Emerging therapies in hematologic malignancies symposium, Super Friday Symposium, American Society of Hematology annual meeting, San Diego CA
2005	“New developments in lymphoma therapy”, Hematology/Oncology Grand Rounds, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx NY
2005	“New developments in immunotherapy for NHL”, Hematology/ Oncology Research Conference, Mount Sinai School of Medicine, New York, NY
2005	“New developments in radioimmunotherapy for lymphoma”, Winter Oncology Conference, Whistler, British Columbia, Canada
2005	“Mantle cell lymphoma”, Winter Oncology Conference, Whistler, British Columbia, Canada
2005	“New developments in lymphoma therapy: Can we get rid of chemotherapy?”, Medical Grand Rounds, Weill Medical College of Cornell University, New York Presbyterian Hospital
2005	“Epratuzumab in aggressive NHL”, Lymphoma…the next questions conference, Fort Lauderdale FL

2005	"New developments in lymphoma therapy", Hematology/Oncology Grand Rounds, University of Rochester School of Medicine
2005	"Novel strategies to enhance chemoimmunotherapy for NHL", Section of Hematology/Oncology, University of Nebraska Medical Center
2005	"Follicular Lymphoma", invited discussant, American Society of Clinical Oncology annual meeting, Orlando FL.
2005	Symposium chair, "Radioimmunotherapy: Why should we use it? Why don't we use it?", Pan Pacific Lymphoma Conference, Lihue, HI
2005	"Proteosome inhibition in aggressive lymphoma", Pan Pacific Lymphoma Conference, Lihue, HI
2005	"Radioimmunotherapy for lymphoma", Fifth International Congress on Monoclonal Antibodies in Cancer, Quebec City, Canada
2005	"New developments in lymphoma: can we get rid of chemotherapy?", Columbia University College of Physicians and Surgeons, NY
2005	"Treatment of the elderly patient with indolent lymphoma", Geriatric Oncology Consortium meeting, Washington DC
2005	"Idiotype vaccination for NHL", Chemotherapy Foundation Symposium, New York NY
2005	"Epratuzumab" and "Iodine-131 tositumomab", New drugs in hematologic malignancies meeting, Bologna Italy
2005	Invited speaker, American Society of Clinical Oncology, Medical Oncology Knowledge Workshop, Reston VA
2005	"Idiotype vaccination for aggressive lymphoma", Super Friday Symposium, American Society of Hematology annual meeting, Atlanta GA
2005	Invited speaker, education session, Targeting CD20 in follicular NHL: Novel anti-CD20 therapies, antibody engineering, and the use of radioimmunoconjugates, American Society of Hematology annual meeting, Atlanta GA
2006	"New developments in lymphoma therapy", Hematology/Oncology Grand Rounds, Rush University Medical Center, Chicago IL

2006 "New developments in Y-90 ibritumomab tiuexetan radioimmunotherapy for lymphoma", Winter Oncology Conference, Whistler, British Columbia, Canada

2006 "Risk-adapted initial therapy for follicular lymphoma", Winter Oncology Conference, Whistler, British Columbia, Canada

2006 "New developments in lymphoma therapy", Hematology/Oncology Grand Rounds, Beth Israel Medical Center/St. Lukes-Roosevelt Medical Center, New York, New York

2006 "New developments in antibody therapy: beyond rituximab", Second International Conference on Childhood NHL, New York, NY

2006 "Optimizing treatment options for low-grade lymphoma", CME symposium "Novel target therapies in hematologic malignancies: expanding efficacy and applications", 2006 annual meeting of the American Society of Clinical Oncology, Atlanta GA.

2006 "Monoclonal antibodies in hematologic malignancies", 8^{th} International Congress on Biological Therapy of Cancer" (BDA/ESMO/NCI), Dresden, Germany.

2006 "High-risk asymptomatic follicular lymphoma patients should receive immediate therapy" (debate), Southern Association of Oncology Annual Meeting, Ponte Vedra, FL

2006 "Mantle cell lymphoma", American Society of Hematology State of the Art Symposium, Las Vegas NV

2006 "New developments in lymphoma therapy", Hematology/Oncology Grand Rounds, University of Virginia School of Medicine, Charlottesville, VA

2006 "New developments in radioimmunotherapy of lymphoma", Japanese Society of Hematology/Japanese Society of Clinical Hematology, Fukuoka, Japan

2006 "Recent progress in antibody therapy for lymphoma", Eleventh Conference on Cancer Therapy and Immunoconjugates, Parsippany, NJ

2006 "Radioimmunotherapy for indolent lymphoma", Lymphoma and Myeloma 2006, New York, NY

2006 "New developments in lymphoma therapy", Piedmont Oncology Association, Charleston SC

2006 "Pixantrone", Chemotherapy Foundation Symposium, New York, NY

2006 "Radioimmunotherapy in aggressive lymphoma", Lymphoma in the 21st century, Institute of Hematology and Oncology, University of Barcelona, Spain

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Non-Hodgkin's lymphomas--current status of therapy and future perspectives.

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Non-Hodgkin's lymphomas (NHL) are a heterogeneous group of disorders which can either be classified according to their biology, represented by corresponding counterparts of normal lymphocyte development as in the Kiel classification, or according to their clinical course, used in the Working Formulation. The recently proposed Revised European-American Lymphoma (R.E.A.L.) classification may unify both aspects and facilitate the comparability of international studies. Besides histology, the extent of disease still comprises the major determinant of therapy. In high-grade lymphomas combination chemotherapy with cyclophosphamide, hydroxydaunorubin, vincristine and prednisone (CHOP) represents the treatment of first choice, and may be restricted to 3-4 cycles in patients with limited stages of the disease when followed by involved field radiotherapy. In more extended, bulky stage II to IV disease, treatment must be extended to six courses of CHOP and, potentially, additional irradiation. Even in advanced states of the disease, long-term remission and potential cure are achieved in 30-50% of cases. In low-grade lymphomas, most patients present with advanced stages III and IV for which chemotherapy can be applied with palliative intention only. Hence, a watch-and-wait approach still seems appropriate outside clinical investigations until the disease requires a therapeutic intervention. This consists preferentially of chemotherapy of moderate intensity such as cyclophosphamide, vincristine and prednisone (COP) or prednimustine and mitoxantrone (PmM). In responding patients, maintenance therapy with interferon-alpha is currently being explored and may result in prolongation of disease-free and, possibly also, overall survival. In both high- and low-grade lymphomas, intensification of therapy by myeloablative chemotherapy or combined chemoradiotherapy followed by autologous bone marrow transplantation (ABMT) or peripheral stem cell transplantation provides a promising and potentially curative prospective. In addition, new cytostatic agents such as the purine analogues--fludarabine, chlorodeoxyadenosine and deoxycoformycin--enlarge the therapeutic spectrum. More experimental approaches consist of the application of immunotoxins or radioisotypes, coupled to monoclonal antibodies directed against lymphoma-specific antigens. Overall, the substantial advances that have been achieved in the understanding of the biology and pathogenesis of malignant lymphomas, as well as the current achievements of therapy and the new promising perspectives, justify the hope that curative therapy can soon be offered to an increasing proportion of patients with NHL.

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Non-Hodgkin's lymphomas: current classification and management.

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- **Dorfman DM**.

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Considerable progress has been made in the classification of non-Hodgkin's lymphomas during the past 15 years, and the use of specific monoclonal antibodies directed against cell surface antigens has contributed to the understanding of the immunology of the disease. Early-stage indolent lymphoma is treated with radiotherapy; treatment of advanced-stage indolent lymphoma varies. Aggressive lymphomas are treated with combination chemotherapy with or without regional radiotherapy, and highly aggressive lymphomas are treated with regimens similar to those for children with leukemia.

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1. Quality of Life in Low-Grade Non-Hodgkin's Lymphoma

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David Cella, PhD, *Research Professor, Institute for Health Sciences, Research and Policy Studies, Northwestern University, Director, Center on Outcomes, Research and Education, Evanston Northwestern Healthcare*

Reviewer's Comments:

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Low-grade non-Hodgkin's lymphoma (NHL) is an indolent form of the disease with a generally slow course of progression. Although still usually incurable, low-grade disease has shown responsiveness to some of the newer chemotherapeutic and nonchemotherapeutic treatment options. However, since cure remains elusive, and since many patients with low-grade NHL may have few or even no symptoms initially, the decision about whether or not to initiate treatment logically must include quality-of-life (QOL) issues. This paper summarizes clinical and diagnostic characteristics of low-grade NHL that have some bearing on QOL considerations. Adverse effects of the more common treatment approaches are discussed according to their QOL implications, illustrating the relevance of QOL to the clinical management of low-grade disease. Finally, data from an ongoing study using the Functional Assessment of Cancer Therapy (FACT) measurement system are presented. These data offer a basis for comparing the QOL of patients with NHL to

that of individuals with other solid tumors, and also illustrate the effects of chemotherapy on QOL.[ONCOLOGY 12(5): 697-717, 1998]

In 1997, an estimated 54,000 people in the United States were diagnosed with non-Hodgkin's lymphoma (NHL).[1] This disease thus accounted for nearly 4% of cancer incidence overall.[1] In the same year (1997), almost 24,000 people died of the disease.[1]

The subclassification of low-grade NHL constitutes approximately 25% of all cases.[2] Incidence of low-grade disease is higher in people between the ages of 35 and 64 years (37%) than in those under age 35 (16%).[3] Ironically termed the "favorable" or indolent form of NHL due to a natural history characterized by slow disease progression and a relatively long duration of survival (7 to 10 years)[4] when compared to intermediate- and high-grade disease, low-grade NHL is still regarded as essentially incurable.[5,6]

The addition of new treatment options, a better understanding of factors that predict response, and the introduction of new nonchemotherapeutic therapies have improved the clinical management of low-grade NHL. These improvements have not yet produced a significant increase in cure rate, however.[7-9] For this reason, and because life-extending treatments produce toxicity and added cost, quality of life (QOL) emerges as a very relevant consideration when judging therapeutic benefit.

The course of progressive low-grade NHL is typified by sequential remissions and relapses, disease dissemination, and eventual resistance to current treatment approaches.[6] Also, since patients often opt for alternative treatments at times of relapse, they are likely to endure acute and chronic treatment toxicity, as well as psychosocial sequelae associated with chronic, life-threatening disease.

In summary, given that the disease produces symptoms, the chance for cure is low, and available treatments have a questionable impact on survival and known toxicity (or cost), QOL may be the most important clinical management concern. To date, however, there has been a paucity of relevant literature and research on the quality of life of patients with low-grade lymphoma, and no published randomized clinical trial has included QOL evaluation as an outcome. The need for reliable, valid measures of the physical, functional, emotional, and social impact of lymphoma is apparent. Although questionnaires that measure general QOL are available,[10-12] there is no lymphoma-specific QOL questionnaire or subscale that addresses the particular symptoms or concerns of patients with lymphoma or the effects of lymphoma treatments on life quality. Treatment decision-making (by both patient and physician) and practice guidelines would be enhanced by the ability to balance QOL consequences against the known benefits and drawbacks of established and investigational treatments, such as extension of survival time, durability of remission, toxicity of treatment, and effectiveness of palliation.

This paper will summarize the clinical characteristics of low-grade NHL, including classification, staging, and symptoms, as well as the predictors of treatment response. Adverse effects of the most common treatments and their QOL implications will also be discussed, in an attempt to illustrate the high degree of relevance of QOL considerations to clinical management. In addition, the psychosocial sequelae of NHL will be reviewed. Finally, data derived from a commonly used questionnaire, the Functional Assessment of Cancer Therapy (FACT) measurement system,[10] will be presented. These data were used to compare the QOL of patients with NHL to a matched sample of patients with mixed cancer types and a smaller sample of patients with Hodgkin's disease; the QOL of NHL patients according to treatment status (on vs off chemotherapy) were also compared.

2. Clinical Characteristics

Classification and Staging

Non-Hodgkin's lymphomas comprise a wide range of malignancies that originate in the lymphoid system. They differ according to their pathologic and immunologic characteristics and their prognostic classification.[3,5,6] Efforts have been made to create a taxonomy that effectively groups all lymphomas into distinct categories according to their morphology, course, and outcome. Due to the variability of lymphomas, however, development of a pure classification system still remains challenging.[6]

At present, NHLs are commonly classified by the International Working Formulation, established in 1982 by a special task force of the National Cancer Institute to consolidate lymphomas by clinically useful criteria so as to predict biological behavior, curability, and survival.[13] Another classification system, the revised European-American classification of lymphoid neoplasms (REAL) has been proposed but is not universally accepted.[14] Precise diagnostic evaluation of the histologic subtype and classification have become critical for appropriate management of the disease.[6]

The International Working Formulation defines three general categories of lymphomas—low grade, intermediate grade, and high grade—which are differentiated most notably by their aggressiveness or "malignant potential."[5] Low-grade lymphomas include small lymphocytic; follicular, small cleaved cell; and follicular, mixed, small cleaved and large cell subtypes, which are indolent by nature and initially responsive to a variety of treatments but eventually prove nonresponsive.[6]

Non Hodgkin's lymphoma is clinically staged using the Ann Arbor staging classification (stage I, II, III, IV), which indicates the extent to which lymph node regions and extralymphatic sites are involved.[15]

An unusual increase in the incidence of NHL since the 1970s,[5] combined with little improvement in relative survival rates, continues to challenge the medical community,[1] despite the availability of diverse treatment options. Risk factors associated with low-grade lymphomas include increasing age, male gender, exposure to chemotherapy and

radiation, and chronic immunosuppression.[3,5] Of particular importance to QOL are practice guidelines based on poor prognostic factors, such as age.

Diagnosis
Diagnostic procedures to determine the type of lymphoma and extent of disease are quite extensive, and often require that patients undergo a variety of invasive and noninvasive tests, procedures, and surgical explorations. Physical examination, peripheral lymph node biopsies, chest x-rays, computed tomographic (CT) scans, bilateral bone marrow biopsy, spinal taps, blood tests, and surgical evaluation of tissue and organ involvement collectively lead to an accurate diagnosis.[3,5]

Repeat assessments are necessary to determine response to treatment and guide therapeutic decisions. Chronic invasive assessments in patients with hematologic malignancies cause discomfort and are associated with heightened anxiety, especially at follow-up visits, where fear of recurrence may be confirmed.[16]

Symptoms
Disease symptoms include both common lymphoma symptoms that are indicative of active disease and specific symptoms highly influenced by the location(s) and extent of disease dissemination.[5] The majority of patients present initially with asymptomatic adenopathy (lymph node swelling) and may have active disease without symptoms for up to 3 years after diagnosis, making early treatment (for some) optional.[17] The indolent nature of the low-grade subtypes may also allow some patients to live a relatively prolonged symptom-free and active life until the disease progresses.

Nonspecific lymphoma symptoms, commonly referred to as B symptoms, include fatigue, fever, weight loss, and drenching night sweats. These symptoms are prognostically unfavorable and therefore are often an indication for treatment.[18] Other relevant symptoms include pain and cosmetic problems due to enlarged lymph nodes.

Independent of prognostic differences, treatment of symptomatic disease is more easily justifiable than treatment of asymptomatic disease, on QOL grounds. This will be addressed below.

Site-specific involvement can be limited or widespread, can occur anywhere in the lymphatic system (eg, lymph nodes, spleen, and bone marrow), and can spread to one or more extralymphatic organs (eg, stomach, intestine, bone, skin, oral cavity, and pharynx).[3,5] The presence of bulky masses causes discomfort and often pain.

Other symptoms vary and may include abdominal pain, ulcers, or bleeding if the gastrointestinal (GI) tract is involved.[5] If there is throat or sinus involvement, head and neck discomfort, throat pain, or swallowing difficulty can occur.[5] Patients with neurologic or musculoskeletal system involvement may experience neurologic and musculoskeletal pain and muscle weakness. With bone marrow involvement, weakened immunity or chronic infections can develop.[3]

In short, the range of possible symptoms and functional problems associated with low-grade NHL is diverse, and depends on the site and degree of involvement. Progressive disease dissemination to additional sites places patients at increased risk for new symptoms and problems, which are often unpredictable, although manageable with palliative therapies.

3. Predictors of Treatment Response and Survival

A great deal of attention has focused on evaluating predictors of treatment response and overall survival in order to optimize treatment selection.[18-22] Histologic subtype, disease stage, prior treatment, and age are often cited as determinants of treatment selection.[7,23,24] The presence of B symptoms, age greater than 65 years, poor performance status, high serum lactic dehydrogenase (LDH) levels, number of nodes involved, number of sites involved, intraabdominal involvement, immunoglobulin level, advanced (stage III/IV) disease, and histologic transformation to intermediate- or high-grade disease have been identified as factors that adversely affect survival.[18-22] These factors may also have important QOL implications, especially if they contribute to a significant decrease in physical well-being and functioning or preclude further treatment.

Histologic transformation to intermediate- or high-grade lymphoma has been observed in the natural course of untreated lymphoma and in patients who have received prior therapy.[17] Generally, transformation to higher-grade disease is an unfavorable sign, although there is a subset of patients who have responded well to additional therapy and have enjoyed relatively long-term survival.[25] Fear of histologic transformation (increasing aggressiveness and potential fatality of the disease) is an uncertainty that many patients with low-grade lymphoma must face.

In addition, the risk of developing a second malignancy has been shown to be higher (21%) in long-term NHL survivors (3 to 20 years), as compared with the estimated cumulative risk in the general population (15%).[26] This increased risk is believed to be related, in part, to immunosuppression and exposure to radiation therapy and chemotherapy.[25]

4. Treatment Options

Treatment of low-grade lymphoma continues to challenge physicians, who essentially are managing a chronic, incurable disease over many years. With the exception of some patients with localized stage I lymphomas treated with radiation and some stage II patients treated with combined chemotherapy and radiation, to date there is little evidence confirming that the type of treatment administered has a significant bearing on overall survival.[9,27] In addition, the advantages of early or aggressive treatment and its impact on overall survival have not been clearly demonstrated.[28,29] However, several treatment options are available with varying toxicity and QOL trade-offs: radiation therapy, alone or combined with chemotherapy, single-agent chemotherapy (oral and injectable), combination chemotherapies, bone marrow transplantation (BMT), monoclonal antibodies, and maintenance therapies.

QOL Implications

Standard treatment guidelines for low-grade lymphomas have been difficult to create given the diversity and relative safety and efficacy of available treatments, the wide range of currently identified prognostic factors, and the opportunity for participation in new clinical trials.[7] Although patients may benefit from many therapies, selecting optimal treatment approaches throughout the course of the disease can involve risks (perceived and actual) and trade-offs. For example, patients whose lymphoma is not treated initially may feel uneasy about not actively fighting the disease. Conversely, patients who pursue initial aggressive treatment may endure debilitating, even life-threatening side effects with long-term QOL consequences for an unknown potential advantage.

The lack of clinical trial data on QOL creates obstacles to deciding among various treatment options, especially with regard to early or aggressive therapies that have not been shown to benefit the traditionally most important outcome (overall survival). The degree to which patients are involved in this decision-making process is unclear, and probably depends somewhat on patient attitude and pursuit of information, as well as physician approach, ability, and interest in summarizing and communicating information about the trade-offs between treatment toxicities and probability of benefit. Although it is often helpful for patients to research and learn about their disease and exercise control over treatment choices, repeated involvement and informed consent to treatments that essentially involve a progressive gamble can be emotionally burdensome.[16]

Watchful Waiting vs Active Treatment—The "watch-and-wait" approach established by the Stanford group in the early 1980s[17] is a conservative approach to the treatment of a select group of patients with newly diagnosed low-grade NHL. Investigators have shown that watchful waiting for disease progression or symptoms before initiating therapy does not adversely affect overall survival.[17] For some patients, a high level of physical and functional QOL may be maintained for 3 or more years due to the fact that they are asymptomatic, may have spontaneous disease regression, and are not subject to the toxicities of induction or maintenance therapies.[17] However, because these clinically logical conclusions were not demonstrated with formal QOL assessment, little is known about the emotional and social well-being of patients who defer treatment.

Quality-of-life implications for patients receiving active treatment depend, in part, on the type of therapy, method and frequency of delivery, and the availability and use of supportive agents to counter side effects. Frequently, chemotherapy for NHL is myelotoxic, compromising an already deficient immune system. The addition of radiation therapy, other alkylating agents, or biological response modifiers can significantly contribute to the problem. For example, the combination of chemotherapy and total-body irradiation may lead to acute bone marrow suppression,[30] and the addition of interferon to chemotherapy may result in debilitating side effects, such as fatigue.[31]

Because there are no formal QOL data from randomized clinical trials of low-grade lymphoma patients, we can only estimate the impact of previously studied treatments on QOL by treating toxicity data as a proxy for QOL assessment. Naturally, this must be done with caution because the provider is the source of toxicity data, whereas the patient

is the source of QOL data. Also, toxicity data cover only *some* of the treatment-related QOL problems that patients can have.

Radiation Therapy
Radiation therapy has been shown to produce significant, possibly curative results in a subgroup of patients with early-stage (I or II) localized low-grade lymphoma.[27] Except for palliative reduction of bulky disease, radiation therapy is not commonly used in the management of advanced disease. Localized radiotherapy (involved-field, extended-field, or total lymphoid irradiation) usually does not yield serious toxicities. Skin sensitivity and dryness are common problems at local sites.[32]

When combined with chemotherapy in any stage of disease, radiation therapy may cause pneumonitis, myocardial toxicity,[30] side effects specific to the radiation site,[32] gonadal dysfunction, and sterility.[33] When radiation therapy is used for palliative reduction of bulky disease, its value can be understood as a trade-off between symptomatic relief and treatment toxicity, since survival is not altered.

Chemotherapy

Both single chemotherapeutic agents and multidrug combinations have been used in the treatment of low-grade NHL.

Single Agents—Single-agent therapies, such as chlorambucil (Leukeran) and cyclophosphamide (Cytoxan, Neosar), have been shown to yield similar responses to more aggressive multidrug regimens in their impact on overall survival and are generally less toxic.[34,35] In clinical trials of each drug, most patients experienced treatment-related toxicities, including nausea/vomiting/anorexia and/or diarrhea (31%),[35] GI symptoms (90%),[34] and leukopenia (58% with cyclophosphamide[34] and 67% with chlorambucil[35]). Thrombocytopenia (43%) was reported with chlorambucil,[35] while hemorrhagic cystitis (37%) and alopecia (26%) were observed with cyclophosphamide.[34]

Combination regimens—such as CVP (cyclophosphamide, vincristine [Oncovin], and prednisone) and CHOP (cyclophosphamide, doxorubicin, Oncovin, and prednisone) often produce more toxic effects, and are accompanied by questionable trade-offs. For example, aggressive front-line treatment does not extend overall survival but has been shown to induce a more rapid response, higher response rate, and longer freedom from disease progression.[28,29,36] Data from trials of CHOP report major hematologic and neurotoxic effects, however, as well as death secondary to therapy.[37,38] Two clinical trials reported fatal toxicities in 1%[37] and 3%[38] of patients and grade 3 or 4 toxicities in 28%[38] and 31%[37]. In other trials, common side effects of CHOP included leukopenia (89% grade 3 or 4), thrombocytopenia (11% grade 3), and anemia (74% grade 3). Neutropenic fevers were common and often led to hospitalization (47%).[39]

Low sperm count and increased risk of infertility in men have also been shown to be consequences of combination chemotherapy.[40] The combination of cisplatin (Platinol)

and etoposide yielded serious toxicities when administered to 51 patients; these included hematologic toxicity (39% neutropenia, 35% thrombocytopenia, 16% anemia), severe hemorrhage,[3] and one death due to infection.[41]

Newer aggressive multidrug regimens used in patients with relapsed low-grade lymphoma, such as FMD (fludarabine, mitoxantrone, and dexamethasone) and DHAP (dexamethasone, Ara-C, and Platinol), have been shown to cause severe myelosuppression and secondary opportunistic infections (such as herpes zoster and *Pneumocystis* infections), bacterial and fungal infections, renal insufficiency, mucositis, and neurologic toxicity.[42,43] Some of these treatments have yielded higher response rates than traditional salvage therapies, but not without cost, functional impairment, and life-threatening toxicity.

Purine Analogs—The relatively new purine analogs have shown promise in low-grade lymphoma and thus provide additional options and challenges for the management of this disease.[44] Fludarabine (Fludara), 2'-deoxycoformycin (pentostatin [Nipent]), and 2-chlorodeoxyadenosine (2-CDA, cladribine [Leustatin]) have demonstrated single-agent antitumor activity, with documented partial and complete response rates and prolonged remissions.[45,46]

The frequency and degree of toxicity reported vary by trial but are consistent with the hematologic side effects of other chemotherapies, and include lymphopenia, leukopenia, neutropenia, thrombocytopenia, opportunistic infections, bacterial and fungal infections, nausea, diarrhea, and peripheral neuropathy.[46-48] Infection-related deaths secondary to treatment have been documented with both fludarabine and cladribine.[48-50] One recent study suggested that dose reductions of standard cladribine treatment can afford patients similar results without the severity of myelotoxicity and infection risk that accompany elevated dose—a meaningful QOL consideration.[51]

The purine analogs are important additions to the spectrum of treatment options. These agents have received a great deal of attention recently due not only to their value as effective anticancer drugs but also to their ability to invoke remissions in previously treated and older patients.[47]

Bone Marrow Transplantation

Interest in evaluating the success of autologous and allogeneic BMT for patients with low-grade lymphoma has paralleled advances in the science of stem-cell transplantation. As is true for most other available treatments, stem-cell transplantation has been shown to prolong failure-free survival, but more mature data are needed to demonstrate any overall survival advantage or curative effect.[52-56] Severe, life-threatening toxicities of myeloablative chemotherapy and radiation therapy are considerable. In one retrospective review of low-grade lymphoma patients, 8% died within 100 days of transplantation.[56]

The QOL consequence of BMT have been studied in patients with mixed cancers (including those with NHL). Although many patients have a good recovery from BMT, the period of convalescence is protracted and may include a prolonged hospital stay in

isolation. This can precipitate long-term physical and functional problems. Treatments can also cause gonadal damage and infertility (with their attendant consequences for psychosocial and sexual functioning).[33]

In one study of allogeneic transplantation, 40% of recipients took more than 1 year to return to normal physical and psychosocial functioning and employment status.[57] Another study of patients receiving an allogeneic or autologous transplant demonstrated that a majority of patients experienced physical difficulties (weakness and fatigue), as well as sexual and occupational problems, at more than 12 months post-BMT.[58]

Without demonstrated cure, treatments as intensive as BMT pose a compelling QOL trade-off challenge to low-grade lymphoma patients and their providers with regard to making treatment decisions.

Monoclonal Antibodies

New treatment approaches for low-grade lymphoma include monoclonal antibodies that attach to receptors found on B-lymphocytes. One general approach uses radiolabeled antibodies; another uses a "naked" antibody. Preliminary studies of these monoclonal antibodies as single agents has demonstrated encouraging response rates and some evidence of long-term disease control, but the median duration of response and impact on overall survival are still unknown.[59]

The most frequently observed side effects of monoclonal antibody therapy occur during the infusion and include rigor, fever, chills, nausea, headaches, and hypotension.[60,61] The infusion-related side effects typically diminish in severity with repeated administration.[60,61] Other toxicities include myelosuppression and infections, which occur at a lower rate with naked antibodies than with radiolabeled antibodies.[60,61]

Given the available spectrum of chemotherapy regimens and their toxicities, treatments that have limited and non-life-threatening toxicities, such as monoclonal antibodies, may be relatively more favorable to the QOL of patients who endure repeated treatments and relapses.

Maintenance Therapies

Maintenance therapies are also used in the treatment of low-grade lymphoma, and are believed to contribute to the prolongation of relapse-free survival—a meaningful end point given the incurability of the disease.[62,63] Maintenance therapies, such as interferon- alfa-2a (Roferon-A) or interferon-alfa-2b (Intron A) and intermittent CVP, have yielded mixed results with regard to their value and efficacy. Although progression-free survival advantages have been demonstrated, the trade-off of chronic side effects makes the decision of whether to use maintenance therapy a difficult one.

Leukopenia, thrombocytopenia, anemia, vomiting, and neurologic effects were seen in one trial of CVP.[62] Some patients (14%) withdrew from the study and an additional 14% refused further treatment because of side effects.[62]

Interferon as maintenance therapy has also had varied results but is known to cause debilitating fatigue and other flu-like symptoms, which may be of severe consequence to physical well-being and role functioning.[64,65] One study of multiple myeloma patients who received interferon therapy demonstrated that side effects had negative QOL consequences (ie, fatigue and fever had a negative impact on functioning), but for a subgroup of patients, the decrease in life quality was worth even a small survival or disease-free survival benefit.[31]

This finding provides a good argument for the value of further QOL research for treatments (such as interferon) complicated by trade-offs that may vary according to patient preferences. The integration of QOL data and patient preferences, combined with the evaluation of trade-offs, such as with the Quality-Adjusted Time Without Symptoms or Toxicity (Q-TWiST) statistical technique, may further guide the choice of optimal treatment. The concept of a "symptom-free interval," if developed within the Q-TWiST methodology to include patients with active, essentially asymptomatic disease, could help move this effort forward.

Overall, balancing treatment efficacy and toxicity is important in determining its value. When cure is unlikely or impossible, extending life becomes a valued goal. A treatment that extends life without improving its quality relative to a no-treatment alternative becomes increasingly less valuable as treatment toxicity becomes increasingly more significant.

5. Psychosocial Sequelae of NHL

A great deal of literature is available on the QOL and psychosocial experience of cancer treatment and survivorship, but little pertains specifically to low-grade lymphoma. Hodgkin's disease has offered investigators a unique opportunity to study QOL and long-term psychosocial adaptation of a group of patients in which the cure rate approaches 90%.[66,67] Quality-of-life research on other tumors, such as lung cancer, provides important information about treatment differences and may have value as a predictive indicator of survival and response to therapy.[68] It has been suggested that this information can be incorporated into practice guidelines to help guide decisions about whether to continue aggressive therapy or switch to palliative care.[68] Patients with low-grade lymphoma are at risk of undergoing repeated aggressive and experimental treatment approaches with questionable trade-offs, may live for protracted periods of time as "survivors" (free of disease), are likely to experience difficulties in psychosocial adaptation to illness and to long-term survivorship, and must contend with the uncertainty of relapses and essential incurability of the disease.

QOL Concerns of Patients With Hematologic Cancers

Lesko[16] has comprehensively reviewed the QOL concerns of patients with hematologic malignancies, including issues common to the cancer experience and those specific to leukemia and lymphoma. Due to the potential fatality of the disease and prolonged, complicated treatment approaches, these patients have heightened concern about issues of death, dependence, disfigurement, disruption, and disability. Other significant concerns

related to living with uncertainty and the "emotional exhaustion" due to the potential long-term clinical course of the disease, financial burden, increased risk for depressive mood and anxiety, and family disruption.

Long-term survival issues included conditioned nausea and vomiting, medical concerns (long-term and late effects of treatment), and psychological concerns, such as fear of recurrence and abandoning the role of patient. Conditioned nausea and vomiting, for example, have been shown to extend 7 to 12 years beyond treatment in patients with Hodgkin's disease.[69]

Anxiety and Depression—Other research has demonstrated the prevalence of depression and anxiety in mixed lymphoma patients.[70-72] In one study of the prevalence of psychological distress in 2,388 patients with various types of cancer (breast, lung, colon, head and neck, gynecologic, prostate, and brain cancers, lymphoma, hepatoma), lymphoma patients scored highest for depression.[70]

A high level of anxiety and depressive symptoms or illness (49%) was also found in a group of 98 patients with Hodgkin's disease or NHL.[72] Mood disturbance was associated with negative treatment effects (particularly pain and changes in appetite and taste). Common adverse effects of treatment included hair loss, vomiting, nausea, and loss of appetite. Quality-of-life effects of treatment-related emesis impaired the ability of some patients to complete tasks, work, care for themselves, perform normal daily activities, and enjoy social activities and meals. Following treatment, a subgroup of patients continued to experience a lack of energy, loss of libido, irritability, tiredness, and thinking/memory handicaps. Impairments in social adaptation were less well-defined, although patients reported problems or long-term delays in returning to work.[72]

Symptoms of depression and anxiety over the impact of disease on health and life expectancy predominated in 40 mixed lymphoma patients.[73] Psychological status did not change significantly over the course of early treatment,[73] although other evidence has suggested that psychological symptoms at diagnosis are more severe than at 4 months post-diagnosis.[74] Psychological adaptation in patients with chronic illness may be pivotal to coping with the physical, emotional, social, and functional disrup-tions caused by cancer, including lymphoma.[74,75]

Uncertainty about the illness— has been cited as a psychosocial issue in patients with hematologic malignancies.[76] Unfortunately, available data relate to other diseases. One study of women with gynecologic cancers suggests that uncertainty about the illness-wellness state is predictive of some health-related QOL scores.[77] Low-grade lymphoma patients may be at high risk for the negative implications of uncertainty, and may benefit from research evaluating the impact of uncertainty in illness, as well the value of implementing interventions designed to help patients cope with uncertainty and fear of recurrence or progression.

In a group of 40 mixed cancer patients (including lymphoma patients), the negative impact of cancer recurrence has been shown to be considerable, often distinguished as

being more traumatic than the initial diagnosis.[78] Risk of emotional trauma at points of recurrence are likely to be substantial in patients with low-grade lymphoma. Loss of control (physical and psychologically based), feelings of grief and anger, presence of chronic fatigue, and other long-term and late effects (such as permanent central nervous system damage and infertility), altered self-esteem and self-image, difficulties with resumption of prediagnosis roles, somatic fixation at disease-free intervals, employment and insurance discrimination, and family disruption have also been addressed,[16,76,79-81] but deserve further attention. Table 1 lists the major QOL issues that may concern patients with low-grade lymphoma.

Positive Adaptation to Illness—Low-grade lymphoma patients may suffer diverse treatment and psychosocial consequences, with a select group able to live a relatively healthy and active lifestyle for a considerable time following diagnosis. Some patients will be exposed to aggressive and experimental therapies, while others, especially the elderly, may endure a less toxic, less debilitating course of treatment.

Psychosocial adaptation to illness and survival seem to be highly influenced by a host of variables and, therefore, are likely to vary from patient to patient. Positive aspects of living with chronic or terminal illness have been documented and should not be overlooked in this population. Social, family, and emotional domains have been shown to be positively influenced by life-threatening illness.[75,82] The ability to find meaning in illness, "dispositional optimism," and hope are thought to mediate this effect.[82] Patients may report an increased awareness and enjoyment of "life in the moment" or find value in such things as the coping skills developed by children who have lived with a parent who has a chronic illness or who have faced parental loss.[83]

6. QOL Evaluation of Patients With NHL

An understanding of the overall QOL of people with NHL can be achieved by combining the medical/physical aspects of the disease (described earlier) with its psychosocial sequelae. Taken together, the physical, functional, psychological, and social impact of NHL and its treatment can be evaluated using one of the currently available QOL questionnaires created for people living with cancer. One such questionnaire, the Functional Assessment of Cancer Therapy-General (FACT-G),[10] has been administered in an ongoing study to over 1,000 cancer patients, many of whom have NHL. Some of these data will be presented below.

NHL Patients Compared to Other Cancer Patients

Table 2 presents QOL (FACT-G) data from a mixed sample of 141 patients (inpatients and outpatients) with NHL. These patients represent a subset of a larger sample of 1,196 patients with mixed cancers from five institutions (Rush-Presbyterian-St. Luke's Medical Center, Northwestern University, Medical College of Ohio, Fox Chase Cancer Center, and Johns Hopkins Oncology Center). The mean FACT-G scores are quite comparable to those of the original standardization sample.[10]

To allow a direct comparison of FACT subscale scores between NHL patients and patients with general solid tumors from the same study, an age- and gender-matched group of 141 general cancer patients was extracted. This comparison showed no significant differences on any of the FACT subscales, suggesting that the QOL of NHL patients is generally comparable to that of patients with other cancers.

To examine the comparability of patients with NHL to patients with other lymphomas (ie, Hodgkin's disease), a subset of 32 NHL patients, again matched for age and gender to the existing set of 32 Hodgkin's disease patients, was extracted. This comparison, unlike the previous one, showed that the NHL patients reported significantly lower physical and functional well-being than the Hodgkin's disease patients (see Table 3). These lower scores resulted in decreased FACT-G (total QOL) scores as well.

To follow up on this difference, a multiple regression analysis was conducted on the matched groups, entering five predictors of FACT physical well-being and functional well-being scores: diagnosis; Eastern Cooperative Oncology Group (ECOG) performance status; stage of disease; current radiotherapy; and current chemotherapy. The purpose of this analysis was to help determine the extent to which each of these variables contributes to the decline in QOL seen among NHL patients (Table 4). For this reason, all of the factors were entered simultaneously using a stepwise approach, followed by relevant analysis of covariance to confirm unique explanatory ability where noted.

In the first (physical well-being) model, performance status and current chemotherapy were both strongly predictive of physical well-being score, with the five-variable model explaining 48% of its variance. Interestingly, diagnosis itself and stage of disease were not significant factors. Current radiotherapy may have been a nonfactor due to the very low number of patients currently receiving that mode of therapy (5 out of 64). In the second (functional well-being) model, only diagnosis (Hodgkin's disease vs NHL) was predictive of functional well-being score, and the predictive ability was weak overall, explaining only 17% of its variance.

The wording of items in each of the subscales (Table 5) may shed some light on why physical well-being scores were so much more responsive to the effects of chemotherapy. First, all of the items in the physical well-being questionnaire are negatively worded, reflecting the perceived physical state. In contrast, the functional well-being questions are positively worded and emphasize functional ability, which may be less directly and less strongly compromised compared to physical symptoms. A second possible reason is that the physical well-being subscale specifically asks about treatment side effects.

In any event, it is clear from Table 4 not only that physical well-being is very strongly related to performance status but also that, independent of this

relationship, the physical well-being component of QOL is worsened by chemotherapy. This appears to be a more salient factor than age, gender, or actual type of lymphoma (Hodgkin's disease vs NHL).

NHL Patients Who Did and Did Not Receive Chemotherapy

This observation led us back to our original complete set of 141 NHL patients, which we divided into those who received chemotherapy vs those did not (Table 6). A comparison of these two groups showed that patients receiving chemotherapy showed significantly lower physical, emotional, and functional well-being, as well as lower total QOL scores. These differences, presented in Table 6 as unadjusted mean differences for the sake of easy comparison to other samples of patients, remained statistically significant after adjustments were made for other covariates (age, gender, performance status, and stage of disease).

These data provide some empirical support for something that is obvious to most clinicians; namely, that in the absence of a possible direct benefit on disease symptoms, chemotherapy is detrimental to short-term QOL. More importantly, these data provide a basis for comparison to other patient groups, and evidence that the QOL metric produced by the FACT can produce useful, responsive scores in this population. This is not to say that a lymphoma-specific subscale would not be a useful complement to the FACT-G, particularly for the purpose of obtaining lymphoma-specific information related to B symptoms, unique treatment concerns, and strategies for coping with a chronic disease. Such a subscale probably would add a dimension of sensitivity to the assessment.

Comparison of Two Groups Receiving Chemotherapy

As an illustration of how these data, as described, can be used in comparisons with other patient samples, we drew data from a different study of mixed cancer patients (227 of whom had NHL and 46, Hodgkin's disease; sample 2 in Table 7). The purpose of this analysis was not to make a direct statistical comparison between the groups, but rather, to present scores side-by-side to allow rough comparisons to be made. This sample of patients was drawn from a community phase IV trial of epoetin alfa (Epogen, Procrit) for the treatment of anemia related to chemotherapy. All of these patients were, therefore, receiving chemotherapy and had hemoglobin levels below 11 g/dL. Thus, the group of 80 NHL patients receiving chemotherapy from Table 6 would provide a useful comparison (sample 1 in Table 7).

Visual comparison of the NHL patients in sample 1 and sample 2 reveals strikingly similar unadjusted mean scores. On the other hand, the sample 2 NHL patients appear to have higher QOL than the sample 2 Hodgkin's disease patients, in contrast to the sample 1 data presented earlier. However, these differences are not significant after the effects of age and gender are removed.

It has commonly been observed that, under conditions of similar objective health data, older patients tend to report better emotional and even physical well-being. This highlights the importance of considering nondisease factors when comparing QOL data across samples. It is not at all unusual to find that, although different diseases have very unique natural histories, symptoms, and treatment considerations, the QOL considerations and differences may be more strongly related to such factors as age and whether or not the patient is currently receiving treatment.

7. Conclusions

Taken as a whole, the data presented here support a long-held clinical notion about NHL: Given the questionable survival benefit of most treatment options, and given the known adverse impact of chemotherapy on QOL, it seems prudent to manage patients with less aggressive approaches when possible. The presence or absence of disease symptoms is often the determining factor when deciding about treatment, with treatment more likely when symptoms are present. It appears reasonable to conclude that the adverse effects of toxic treatments (ie, cytotoxic chemotherapy) are not easily offset by improvements in clinical outcome or the emotional advantages of receiving treatment, although this conclusion is based on observational data in uncontrolled clinical settings. Therefore, a watch-and-wait or minimally toxic approach to the managements of some patients with low-grade NHL seems to be well-justified. Of course, patient values and preferences for aggressive therapy must be factored into this general equation.

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9. The Webster/Cella Article Reviewed

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Cancer treatment often has debilitating effects on the patients who receive it. Chemotherapy regimens can produce toxicities, such as gastrointestinal disturbances, hematologic deficiencies, fatigue, and neurotoxicity. Patients typically undergo these chemotherapy regimens to increase their disease-free survival time. Given that these therapies can negatively affect a patient's quality of life (QOL), treatments need to provide clear curative potential and/or survival benefits to offset detrimental effects on QOL.

Webster and Cella describe a case in which the benefits exchanged for the risks of therapy are unclear. As they describe, the therapies for low-grade non-Hodgkin's lymphoma (NHL) do not provide a clear benefit in terms of cure or overall survival. The authors therefore conclude that extensive treatment may not be warranted because of the great impact of these therapies on QOL.

When Are the QOL Consequences of Treatment Important?
We concur with Webster and Cella that QOL should be considered when making treatment decisions. There are several clear-cut circumstances in which the effects of treatment on QOL become an important decision-making tool. For example, the QOL consequences of treatment are particularly significant when the treatments being considered yield similar advantages in survival but have different toxicities.[1] This appears to be the case for the treatment options for low-grade NHL, as described by Webster and Cella.

Multiple treatment options exist, including observation only, that have little effect on overall survival but a highly variable impact on QOL. However, these treatments do appear to differ in how they affect other disease related variables, such as the speed of treatment response and time to recurrence.

Although overall survival is usually considered to be the "bottom line" in evaluating therapies, treatment efficacy in terms of time to recurrence should not be underemphasized. For a patient facing the long-term management of a chronic, incurable disease, the amount of time that he or she remains free of disease or with stable disease may be important. This is particularly true if the disease itself produces debilitating symptoms. However, for patients whose disease does not manifest serious symptoms, as is the case for many individuals with low-grade NHL, the advantage provided by a longer time to progression may be primarily psychological.

According to Webster and Cella, for patients with low-grade NHL, progression is slow, symptoms are few, and therapies offer little survival advantage. Thus, the complexity of QOL considerations must be carefully evaluated.

To date, research on the QOL of patients with low-grade NHL is limited. Even the data presented by Webster and Cella are not specific to low-grade NHL. Thus, more research is needed before strong conclusions can be made about treatment strategies for low-grade NHL that optimize both medical benefit and QOL.

Assessing Patients' Preferences

Although QOL may be compromised as the result of treatment, patients may be willing to endure these difficulties if therapy offers a longer symptom-free interval, or even the remote chance of cure or increased survival. The ambiguity surrounding the appropriateness of treatment for low-grade NHL indicates the need for information on patients' preferences for treatment outcomes, taking into account both changes in QOL and life expectancy.

One group of methods for quantifying patient's desires regarding treatment decisions is the assessment of utilities, or patient preferences. Utilities are used by decision scientists in evaluating treatment options based on a patient's preference for a particular health state.[2] A patient-generated utility is a measure of the patient's perception of the degree of impact of a particular outcome, such as neurologic problems or infertility. Utility is often assessed using such methods as the time trade-off, which seeks to determine the number of years of healthy life that a participant is willing to "trade off" for remaining free of an adverse health condition.

Utilities assess the value of a health state or outcome in reference to a universal standard, such as time, money, or risk of death.[3] This assessment technique is particularly useful when the same adverse event may be perceived differently by different people. These preferences can then be incorporated into formal decision-analytic models to determine the optimal treatment choice.

Need for Psychosocial Interventions

Even when treatment decisions take patient preferences into account, the management of the disease is likely to have consequences for patients' QOL. Patients who receive aggressive treatment must cope with the difficult side effects of chemotherapy. Patients who opt for the strategy of "watchful waiting" may experience stress and anxiety related to not treating the disease.

The impact of low-grade NHL on patients' QOL points to the need for psychosocial interventions to aid psychological adjustment and improve QOL. These programs may be implemented regardless of the treatment strategy chosen.

Several recent reviews have documented the effectiveness of psychosocial interventions in helping cancer patients adjust to their diagnosis and treatment.[4-7] Cognitive and behavioral interventions, such as guided imagery and progressive muscle relaxation, have been found to effectively reduce chemotherapy-induced symptoms and conditioned side effects, such as nausea and vomiting (see Fawzy et al[5]). In addition, cognitive-behavioral and more general support group-based interventions can be effective in improving psychological well-being and increasing overall survival time.[8,9] Group or

individual psychosocial intervention programs can be useful at different stages of disease and treatment, and they need to be made available to all patients.

Although no studies have tested the use of such interventions to help patients cope with the stress of not treating a disease, this could be a fruitful area of research for both low-grade NHL and other cancer sites characterized by slow progression (eg, prostate cancer).

Treatment Risks vs Benefits

Although inclusion of QOL outcomes should be considered when making treatment decisions for diseases such as low-grade NHL, patients' preferences for the trade-off between treatment risks and benefits should also be considered. However, because current methods for assessing utilities are complex and may be impractical in some settings,[3] further methodologic research is needed to improve assessment techniques.

Patients need to be well informed of the consequences of a particular treatment decision, but they also need to be aware of the beneficial effects treatments can have on the disease process. Active involvement in group, individual, and/or personalized psychosocial intervention programs can be a useful adjunct to traditional medical treatment.

Unfortunately, for many patients, these types of programs are not available or affordable. Future research needs to definitively determine the cost-efficacy of these intervention programs so that they can be made available to all patients battling life-threatening illnesses.

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10. The Webster/Cella Article Reviewed

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The management of patients with the less aggressive subtypes of non-Hodgkin's lymphoma remains a clinical challenge. As pointed out by Webster and Cella, this challenge relates, at least in part, to the comparatively long median survival that can be achieved in such patients with a wide variety of treatment approaches. However, it is very important to realize that not all patients with the indolent varieties of non-Hodgkin's lymphoma are the same.

New Classification of Lymphomas
Work by the International Lymphoma Study Group has stimulated a new way of thinking about the classification of the non-Hodgkin's lymphomas.[1] Rather than focus on morphologically defined entities, the emphasis has shifted toward identifying more specific clinicopathologic entities—ie, real diseases. This new approach to classification takes into account biological (ie, genetic, immunologic, and so on) and clinical observations, in addition to cell size, shape, and growth pattern.

The new classification system will allow investigators to focus on specific illnesses for clinical studies. This work has led to the acceptance of a number of "new" lymphomas that previously were unrecognized. These newly recognized entities, such as mantle cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphomas, and anaplastic large cell lymphoma, make up approximately 20% of all non-Hodgkin's lymphomas (Table 1).[2]

Given this new, and I believe improved, approach to labeling lymphomas, the study of "low-grade" non-Hodgkin's lymphomas becomes inappropriate. Rather, studies should focus on small lymphocytic lymphoma, follicular lymphoma, MALT lymphoma, and other specific lymphomas. Each of these illnesses has a different natural history and response to available therapies, and lumping them together risks missing important information.

Quality of Life Issues

The report by Webster and Cella focuses on data generated from patients with follicular lymphoma. This diagnosis represents 20% to 25% of all non-Hodgkin's lymphomas diagnosed worldwide and more than 25% of those diagnosed in North America.[2] It is the best-studied indolent lymphoma and the disease to which most of their comments apply. However, follicular lymphoma is not a uniform disease, and all patients with this diagnosis should not be approached in the same way.

The development of the International Prognostic Index improved clinicians' ability to subcategorize patients with diffuse large cell lymphoma.[3] However, it has become apparent that this system of predicting survival of patients with lymphoma applies to the less aggressive lymphomas as well as the more aggressive subtypes.

Follicular lymphoma is thought to have a good outlook because most patients have favorable risk factors in the International Prognostic Index and a prolonged survival. However, a subset of these patients have a number of adverse risk factors and a survival as poor as any subgroup of patients with aggressive lymphoma.[2] Certainly, these latter patients will require a different management approach.

Quality of life has been a difficult issue to study in patients with cancers, as well as other diseases. However, I believe that some points are clear. One fact that has been surprising to many clinicians is that, when asked, patients seem to value increased survival over any other factor and are willing to accept surprising risks to achieve modest increases in survival. This leads back to the most frustrating question in the management of patients with follicular lymphoma: Does any therapy significantly modify the course of the illness?

Unfortunately, we have no completed, randomized trials to help answer this important question. In particular, no randomized trial comparing therapy to no therapy has been conducted. I doubt that any clinician who cares for patients with lymphoma questions the positive effect of treatment on their clinical course, although treatment can be withheld in many patients until symptoms develop. The debate centers on the timing of therapy and the relative merits of more vs less intensive approaches.

The management of indolent lymphomas, such as follicular lymphoma, is likely to remain a source of controversy for many years. Until definitive answers are available, this field remains one where the art of practicing medicine is still extremely important.

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Treatment of Patients With Low-Grade B-Cell Lymphoma With the Combination of Chimeric Anti-CD20 Monoclonal Antibody and CHOP Chemotherapy

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Purpose: To determine the safety and efficacy of the combination of the chimeric anti-CD20 antibody, Rituxan (Rituximab, IDEC-C2B8; IDEC Pharmaceuticals Corporation, San Diego, CA), and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy.

Patients and Methods: Forty patients with low-grade or follicular B-cell non-Hodgkin's lymphoma received six infusions of Rituxan (375 mg/m^2 per dose) in combination with six doses of CHOP chemotherapy.

Results: The overall response rate was 95% (38 of 40 patients). Twenty-two patients experienced a complete response (55%), 16 patients had a partial response (40%), and two patients, who received no treatment, were classified as nonresponders. Medians for duration of response and time to progression had not been reached after a median observation time of 29 + months. Twenty-eight of 38 assessable patients (74%) continued in remission during this median follow-up period. The most frequent adverse events attributable to CHOP were alopecia (38 patients), neutropenia (31 patients), and fever (23 patients). The most frequent events attributed to Rituxan were fever and chills, observed primarily with the first infusion. No quantifiable immune response to the chimeric antibody was detected. In a subset of 18 patients, the *bcl-2* [t(14;18)] translocation was positive in eight patients; seven of these patients had complete remissions and converted to polymerase chain reaction (PCR) negativity by completion of therapy.

Conclusion: This is the first report demonstrating the safety and efficacy of Rituxan anti-CD20 chimeric antibody in combination with standard-dose systemic chemotherapy in the treatment of indolent B-cell lymphoma. The clinical responses suggest an additive therapeutic benefit for the combination with no significant added toxicity. The conversion of *bcl-2* from positive to negative by PCR in blood and/or marrow suggests possible clearing of minimal residual disease not previously demonstrated by CHOP chemotherapy alone.

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THE NON-HODGKIN'S LYMPHOMAS (NHLs) are a diverse group of lymphoid neoplasms that collectively rank fifth in cancer incidence and mortality.[1,2] The prevalence of NHL has been increasing during the last two decades, and it is estimated that approximately 55,400 new cases and 24,900 deaths will occur in 1998.[2] Recognized since the 1950s as a distinct group of diseases, NHLs range from indolent malignancies (low-grade histologies) to rapidly growing and highly aggressive tumors (high-grade histologies). The overall median age at presentation is 42 years (58 years for low grade), and the incidence increases with advancing age.[3] The majority of NHLs are of B-cell origin,[4] with more than 90% of patients expressing the CD20 antigen.[5]

In general, low-grade or follicular NHL is assumed to have an indolent course when compared with intermediate- and high-grade NHL. Although treatment of low-grade follicular lymphomas with standard chemotherapeutic regimens is characteristically associated with a high initial response rate, the clinical course consists of a pattern of repeated relapse. Subsequent remissions occur, but at a progressively lower rate and with a shorter duration.[6] Patients eventually succumb to the disease or its complications with a median survival of approximately 6.2 years.[7,8] For these reasons, novel therapeutic agents and strategies need to be evaluated in this group of patients.

Molecular research has identified the *bcl*-2 proto-oncogene as being associated with a t(14;18) chromosomal translocation, which has been reported to occur in approximately 50% of NHLs (80% low grade; 30% intermediate grade).[3] This chromosomal translocation moves the *bcl*-2 gene from chromosome 18 to the immunoglobulin heavy-chain locus on chromosome 14 and results in *bcl-2* activation. Resultant overexpression of *bcl-2* protein localizes to the mitochondrial membrane, nuclear membrane, endoplasmic reticulum, and cell membrane.[9] This results in inhibition

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of apoptosis (programmed cell death) and gives cells a survival advantage. B cells containing t(14;18) chromosomal translocation are believed to play a role in lymphomagenesis, possibly serving as the "first hit" in a "multi-hit" theory of carcinogenesis. By use of a sensitive, nested polymerase chain reaction (PCR) assay, cells containing the t(14;18) chromosomal translocation are detectable at an assay sensitivity of one *bcl-2*–positive cell in 10^5 to 10^6 normal cells.[10] In patients with documented B-cell lymphoma, serial analyses of *bcl-2* in blood and marrow by PCR could serve as a method for monitoring minimal residual disease.

Attempts to treat B-cell malignancies with monoclonal antibodies (mAbs) began more than a decade ago with monoclonal antibodies reactive with B-cell antigen idiotypes.[11,12] Customized anti-idiotypic monoclonal antibodies were developed and used alone or in combination with interferon alfa or chlorambucil. Significant clinical activity was observed; however, this type of murine monoclonal antibody therapy was limited by the development of human anti-mouse antibody responses, the relative inability of mouse antibodies to induce human immune effector mechanisms, and the occurrence of idiotype-negative relapses. The technology to alter antibodies genetically by joining the variable region genes of murine antibodies to human immunoglobulin constant region genes allowed the development of a mouse/human chimeric antibody with the demonstrated advantages of reduced immunogenicity and an enhanced ability to interact with human effector cells.

Rituxan (IDEC Pharmaceuticals Corporation, San Diego, CA) is a chimeric monoclonal anti-CD20 antibody that can deplete malignant B cells through complement-dependent cell cytotoxicity, antibody-dependent cell-mediated cytotoxicity,[13] and apoptotic mechanisms. It has also been shown to sensitize drug-resistant lymphoma cell lines to killing by cytotoxic drugs.[14] The monoclonal antibody has shown single agent activity in patients with low-grade or follicular lymphomas. Two previous phase I/II single agent, dose-escalation studies of Rituxan have been conducted in patients with relapsed or recurrent NHL. Fifteen patients were enrolled onto a single dose study (10 to 500 mg/m^2 of Rituxan), and 47 patients were enrolled onto a multiple dose study (125, 250, or 375 mg/m^2 weekly for 4 weeks). Clinical activity was noted in seven of 15 patients in the single dose trial, with two partial responses lasting 8.1 and 8.5 months and five minor responses.[15] In the phase II portion of the multiple dose (375 mg/m^2) Rituxan study, three complete responses and 14 partial responses were noted in 34 assessable patients, with a median response duration of 8.6 months.[16] Median time to progression (TTP) in these responders was 10.2 months; TTP exceeded 20 months in five patients and 30+ months in two patients. Adverse experiences were mostly grade 1 or 2 and consisted primarily of infusion-related events (fever, asthenia, chills, and, less commonly, bronchospasm, hypotension, and angioedema). Hematologic toxicity was usually mild and reversible.

Because of these encouraging results, a phase II open-label, single arm, multicenter study was designed to evaluate the safety and clinical activity of this new monoclonal antibody in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy in the treatment of patients with low-grade B-cell lymphoma. CHOP chemotherapy was chosen because this cytotoxic regimen is an effective first-line therapy for low-grade or follicular NHL. The rationale for the combination of Rituxan and CHOP includes single agent efficacy, non–cross-resistant mechanisms of action, non-overlapping toxicities, and in vitro synergy with certain cytotoxic drugs, including doxorubicin.

PATIENTS AND METHODS

Eligibility

The patient population in this study consisted of newly diagnosed and relapsed/refractory patients at least 18 years of age with histologically documented low-grade or follicular B-cell NHL and measurable progressive disease. Tumors were required to be CD20 positive. Patients were to have an expected survival of 3 months or more; a prestudy performance status of 0, 1, or 2 according to the World Health Organization scale; recovery from any significant toxicity associated with anticancer therapy; and adequate hematologic, renal, and hepatic function within 7 days of initial therapy. The following exclusion criteria applied: "bulky" disease (defined as any single mass > 10 cm in its greatest diameter); prior therapy with anthracyclines, anthrapyrazoles, or drugs that were classified at the time as investigational phase I or II antineoplastic agents; prior radioimmunotherapy; cancer radiotherapy, immunotherapy, or chemotherapy within 3 weeks of the scheduled first study treatment; nitrosourea or mitomycin therapy within 6 weeks of the first scheduled study therapy; or presence of CNS lymphoma. Other exclusion criteria were as follows: significantly impaired organ function, as measured by a serum creatinine level greater than 2.0 mg/dL, a total bilirubin level greater than 2.0 mg/dL, or an AST or alkaline phosphatase level more than 2 times normal; serious nonmalignant disease; active opportunistic infection; major surgery within 4 weeks; and previous or concomitant malignancy other than basal cell or squamous cell carcinoma of the skin, carcinoma-in-situ of the cervix, or other malignancy for which the patient had not been disease-free for at least 5 years. Patients with a New York Heart Association class III or IV heart disease or myocardial infarction within the past 6 months and patients with a left ventricular ejection fraction of less than 45% within 1 month of study enrollment were disqualified from entering onto the study. Patients who had prior anti-CD20 therapy were excluded, except for patients who were previously enrolled onto a clinical trial of Rituxan with negative human anti–chimeric antibody (HACA) serum titers. Patients with a nondemonstrable CD20-positive neoplastic B-cell population in lymph nodes or bone marrow were not included. Pregnant or lactating women and patients of childbearing

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potential, unless using accepted birth control methods, were not allowed to enroll. Eligible patients signed a detailed written informed consent statement meeting the requirements of the institutional review board of the participating institution. Institutional review board approval was given for this study at each participating center.

PCR Assay for t(14;18)

The assay for the detection of cells with the t(14;18) chromosomal translocation by PCR uses a nested primer amplification specifically for either the major breakpoint region or the minor cluster region and was developed at Roswell Park Cancer Institute in collaboration with Dr. John Gribben. The PCR technique used in this study was essentially the same, with minor modifications, as that used and described by Gribben et al.[10] The assay will detect one in 10^5 to one in 10^6 t(14;18)-containing cells for either breakpoint region among a normal background and will discriminate all t(14;18) translocations that occur within these regions. Major breakpoint region- and minor cluster region-positive and -negative cell lines were used as controls. A separate PCR reaction was performed to amplify a region within the *bcl*-2 gene to act as an internal DNA quality control.

Treatment Design

This study consisted of a single treatment group. Patients were to receive a total of six intravenous infusions of 375 mg/m^2 of Rituxan and six cycles of CHOP, given every 3 weeks (Fig 1). Each CHOP cycle consisted of cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, and vincristine 1.4 mg/m^2 (maximum dose, 2.0 mg), given intravenously on day 1, and oral prednisone 100 mg/m^2 on days 1 through 5. Rituxan was produced and supplied as a 5-mg/mL saline solution of antibody in 10-mL glass syringes. The mAb was further diluted in normal saline to a final concentration of 1 mg/mL and administered intravenously through a low-protein-binding 0.22-µm in-line filter. Rituxan infusions 1 and 2 were administered on days 1 and 6 before the first CHOP cycle, which started on day 8. Rituxan infusions 3 and 4 were given 2 days before the third and fifth CHOP cycles, respectively, and infusions 5 and 6 were given on days 134 and 141, respectively, after the sixth CHOP cycle. This mAb schedule was chosen to take advantage of three different characteristics of Rituxan in addition to its known clinical activity in NHL: (1) in vitro data demonstrating its ability to sensitize chemoresistant cell lines; therefore, doses 1 and 2 could be viewed as a form of induction immunotherapy that could possibly render chemoresistant cells chemosensitive; (2) in vitro data demonstrating that possible synergy with cytotoxic agents would best be effected by interim doses 3 and 4; and (3) the generally well-accepted belief that monoclonal antibodies are extremely effective in a minimal residual disease setting; thus, doses 5 and 6 could be viewed as being used as a "mop up" of residual lymphoma after completion of systemic chemotherapy.

If toxicity occurred during the mAb infusion, the infusion was to be slowed or temporarily discontinued and the patients were to be medicated as necessary with acetaminophen (for fever) or diphenhydramine (for rash, mucosal congestion, or other infusion-related reactions) and other medications as needed. Once the adverse events abated, the antibody infusion could be resumed at 50% of the previous rate and then escalated as tolerated. CHOP was to be administered according to the standard preparation and infusion procedures for each institution. If grade 3 neurotoxicity occurred at any time during the treatment period, vincristine could be discontinued at the investigator's discretion. Cyclophosphamide dose modification for hematologic toxicities was to be carried out according to an algorithm provided in the protocol. A patient whose treatment was interrupted for more than 3 weeks for either hematologic or nonhematologic toxicity was to be removed from the study. As stated in the informed consent, patients were allowed to withdraw from the study at any time. Furthermore, treatment was discontinued if disease progression was noted or if, in the opinion of the investigator, it was not in the patient's best interest to continue.

Oral premedication with 650 mg of acetaminophen and 50 to 100 mg of diphenhydramine hydrochloride could be administered 30 to 60 minutes before each mAb infusion. No concurrent antineoplastic therapy was allowed except for localized brain radiotherapy for CNS lymphoma. Surgery that did not affect any sentinel lesions was allowed, and surgery on sentinel lesions was allowed only to detect mAb tumor penetration.

Evaluation

All patients were assessable for the intent-to-treat analysis of tumor response and toxicity. The primary efficacy measure was the response of B-cell lymphoma to treatment as determined by the investigator and confirmed by the sponsor. Lesion evaluations occurred at baseline, before the third cycle of CHOP, after the completion of therapy, and every 4 months thereafter until disease progression (PD) was observed. Any evaluation indicating the onset of a partial response (PR) or complete response (CR) (Table 1) was followed by a confirmatory evaluation no sooner than 28 days later. Ninety-five percent confidence intervals for the response rates were calculated.

Secondary efficacy variables were the TTP and the response duration (PD-free interval). The TTP was measured from the date of the first Rituxan infusion to the date of PD or the date of last contact, whichever was earlier. Response duration was measured from the date of the first

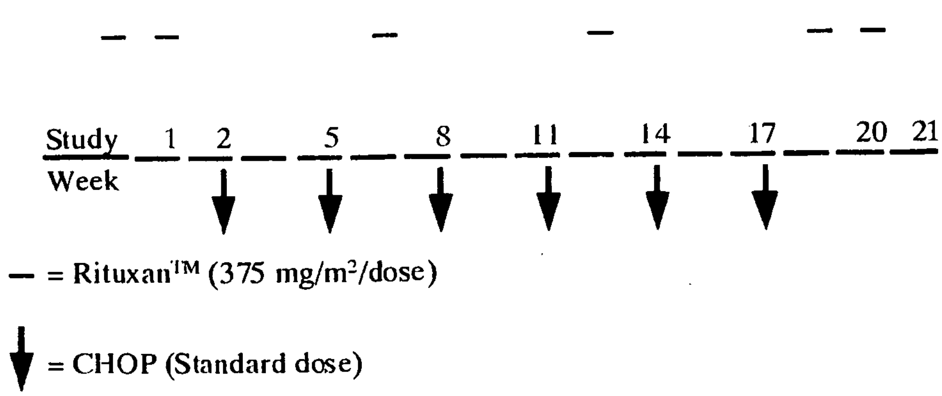

Fig 1. Treatment schedule. Patients received a total of six intravenous infusions of 375 mg/m^2 of Rituxan and six cycles of CHOP given every 3 weeks.

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Table 1. Response Criteria for NHL

Complete response
- No evidence of disease or areas of residual abnormality > 1.0 cm^2 converting from gallium positivity to gallium negativity or proven to be secondary to residual fibrosis by biopsy
- No new lesions
- Confirmed at ≥ 28 days
- Asymptomatic
- No decrease in performance status
- Bone marrow negative (if initially positive)

Partial response
- SPD decrease from baseline ≥ 50%
- No new lesions
- Confirmed at ≥ 28 days

Stable disease
- < 50% decrease in SPD from baseline
- < 50% increase from baseline or nadir
- No new lesions

Progressive disease
- SPD increased ≥ 50% from nadir
- New lesions

Abbreviation: SPD, sum of the products of the perpendicular diameters.

observation of response to the date of PD or the date of last contact, whichever was earlier.

Patients were monitored for the development of an HACA response (samples assayed at the Clinical Immunology Laboratory, IDEC Pharmaceuticals Corporation). Quantitative immunoglobulin levels were also measured at the individual treatment sites.

Each patient's NHL was classified histologically at baseline using International Working Formulation (IWF) criteria, and the stage of each patient's disease was assigned according to the Ann Arbor classification. Pretreatment *bcl-2* [t(14;18)] analysis by PCR was performed in the Laboratory of Molecular Diagnostics at Roswell Park Cancer Institute on peripheral blood and separately pooled bilateral bone marrow aspirate and biopsy samples from 18 patients enrolled onto the study at Roswell Park Cancer Institute. Serial samples for PCR analysis were obtained in those patients testing *bcl-2* positive at baseline.

RESULTS

Patient Demographics and Disposition

The clinical features of the 40 patients (31 previously untreated) enrolled onto this study are listed in Table 2. Thirty-five of the 40 patients received all six infusions of Rituxan and six cycles of CHOP. Two patients were withdrawn from the study before treatment initiation (one patient withdrew for personal reasons, and one patient was withdrawn by the treating physician because of the discovery of lymphomatous involvement of the central nervous system). Three patients discontinued study treatment early (one patient withdrew for personal reasons, one patient withdrew owing to the development of an epidural abscess, and one patient died from reactivation of hepatitis B infection). All 40 patients are included in the intent-to-treat analysis.

Treatment Dose-Intensity

The Rituxan dose was not modified in the 38 patients who received treatment. Doses of one or more chemotherapy agents of the CHOP regimen were adjusted in only 13 patients at some time during the course of the study. In addition, the administration of at least one cycle of CHOP was delayed in six patients by 1 to 3 weeks. Dose-intensity was calculated for cyclophosphamide, doxorubicin, vincristine, and prednisone in each patient as the actual dose received in milligrams per meters squared per week, divided by the calculated total dose in milligrams per meters squared per week. This analysis revealed an average dose-intensity of 0.95 for cyclosphophamide, 0.97 for doxorubicin, 0.95 for vincristine, and 0.95 for prednisone. The average dose-intensity for all of the agents in the CHOP regimen was 0.96 in this chemoimmunotherapy study.

Table 2. Patient Demographics

Characteristic	No.	%
Age, years		
Median	48.5	
Range	29-77	
Sex, male/female	21/19	52.5/47.5
Performance status*		
0	31	78
1	7	18
2	2	5
Time from diagnosis,† years		
Median	0.21	
Range	0.02-8.29	
Histologic grade‡		
A	9	23
B	17	43
C	13	33
D	1	3
Stage§		
I/II	5	13
III	11	28
IV	24	60
Prior lymphoma treatment		
No	31	78
Yes	9	22
Extranodal disease		
No	11	28
Yes	29	72
Elevated serum LDH		
No	29	72
Yes	11	28
Bone marrow involvement		
No	18	45
Yes	22	55

Abbreviation: LDH, lactate dehydrogenase.
*World Health Organization classification.
†Years from diagnosis date to first Rituxan infusion.
‡IWF classification.
§Stage at initial diagnosis.

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Response to Therapy

Median time to response was 47 days (range, 15 to 236 days). The overall response rate to the combination of CHOP and Rituxan treatment was 95% (95% confidence interval, 88% to 100%) in the intent-to-treat patient population (Table 3). Twenty-two patients (55%) experienced a CR, and 16 patients (40%) had a PR. Thus, only the two patients who were withdrawn from the study before the initiation of trial therapy were classified as nonresponders (one with an IWF histologic classification of A and the other, B). Response rates were also evaluated for patient subpopulations, including those with extranodal disease, an elevated serum lactate dehydrogenase concentration, bone marrow involvement, an age of 60 years or more, or bulky disease (Table 3). Combination Rituxan and CHOP therapy achieved at least a partial response in all of the patients, and the complete response rate was less than 45% only for those patients with bulky disease. Twenty-eight (74%) of 38 assessable patients continued in remission after a median observation time of 29 months (Fig 2).

There were 24 assessable (plus one unassessable) newly diagnosed patients with follicular histology, and they all responded to therapy (16 CR and eight PR). The median duration of response was yet to be reached at 27.8+ months. There was no significant difference in duration of response between naive and previously treated patients. There were nine patients with IWF type A histology. Of these, one patient was unassessable, and the other eight patients were responders (three CR and five PR). The median duration of response for the IWF type A patients had not been yet reached at 17+ months. Five of the eight patients were in ongoing remissions. At that time, there was no significant difference in rate or duration of response when these patients were compared with the rest of the study patients.

Eight of the nine patients who had received chemotherapy treatments before study entry responded to therapy with Rituxan and CHOP (Table 4). Five of these responses were complete; two of the complete responses occurred in patients who had experienced either progressive disease or a partial response with their last prior chemotherapy regimen (cyclophosphamide, vincristine, prednisone and chlorambucil, prednisone, respectively).

Table 3. Response to Therapy

		Response					
		CR		PR		CR + PR	
Patient Group	No.	No.	%	No.	%	No.	%
Intent-to-treat	40	22	55	16	40	38	95
Extranodal disease	29	15	52	14	48	29	100
Elevated LDH	11	5	45	6	55	11	100
Bone marrow involvement	22	11	50	11	50	22	100
Age ≥ 60 years	11	5	45	6	55	11	100
Bulky disease*	14	4	29	10	71	14	100

Abbreviation: LDH, lactate dehydrogenase.

*Patients who had a lesion measuring at least 5 cm (but no more than 10 cm) in the largest diameter.

Safety

The most frequently experienced adverse events in this trial were neutropenia (31 patients), alopecia (30 patients), nausea (27 patients), and fever (23 patients). Adverse events that occurred in more than 10% of patients and grade 3 or 4 events are listed in descending order in Table 5. Eight patients were hospitalized with febrile neutropenia and two patients with neutropenia and a documented infection. Most (75%) of the adverse events were attributed to CHOP chemotherapy by the treating physicians. The one death that occurred on study was secondary to reactivation of hepatitis B with resultant hepatic failure and hepatorenal syndrome. Rapid deterioration of liver function tests followed the fifth cycle of CHOP chemotherapy. The most frequent events attributed to mAb treatments were infusion-related events. These events (19% overall) were all grade 1 or 2 in severity and included fever (12 patients), chills (nine patients), and pruritus (six patients). The infusion-related events occurred most frequently with the initial Rituxan infusion and decreased in frequency with subsequent infusions.

A quantifiable immune response to the chimeric antibody HACA was not detected in any patient (limit of detection, 0.57 μg/mL). The mean serum immunoglobulin (Ig) levels for IgG, IgA, and IgM remained within the normal range throughout the trial. Immunoglobulin levels decreased by 50% from baseline and fell below the normal range in 13 patients. Two patients experienced a drop in IgG levels of more than 50% from baseline at days 88 and 146; one (patient no. 27) recovered to normal levels or to within 20% of baseline at day 175. Four patients reported greater than 50% drop from baseline in IgA at days 162, 51, 169, and 97; two recovered to normal levels or to within 20% of baseline at days 92 and 98. Seven patients experienced a drop in IgM levels of more than 50% below baseline; two patients recovered to normal values or to within 20% of baseline at day 176. The immunoglobulin levels for the remaining nine patients had not recovered by the time of the last follow-up examination.

DISCUSSION

Treatment choices for advanced stage low-grade or follicular NHL (IWF type A, B, C, D) have included radiotherapy, single agent chemotherapy, combination chemotherapy, immunotherapy, and combined modalities. Early studies of combination chemotherapy regimens containing anthracyclines resulted in complete response rates of 30% to 40% but

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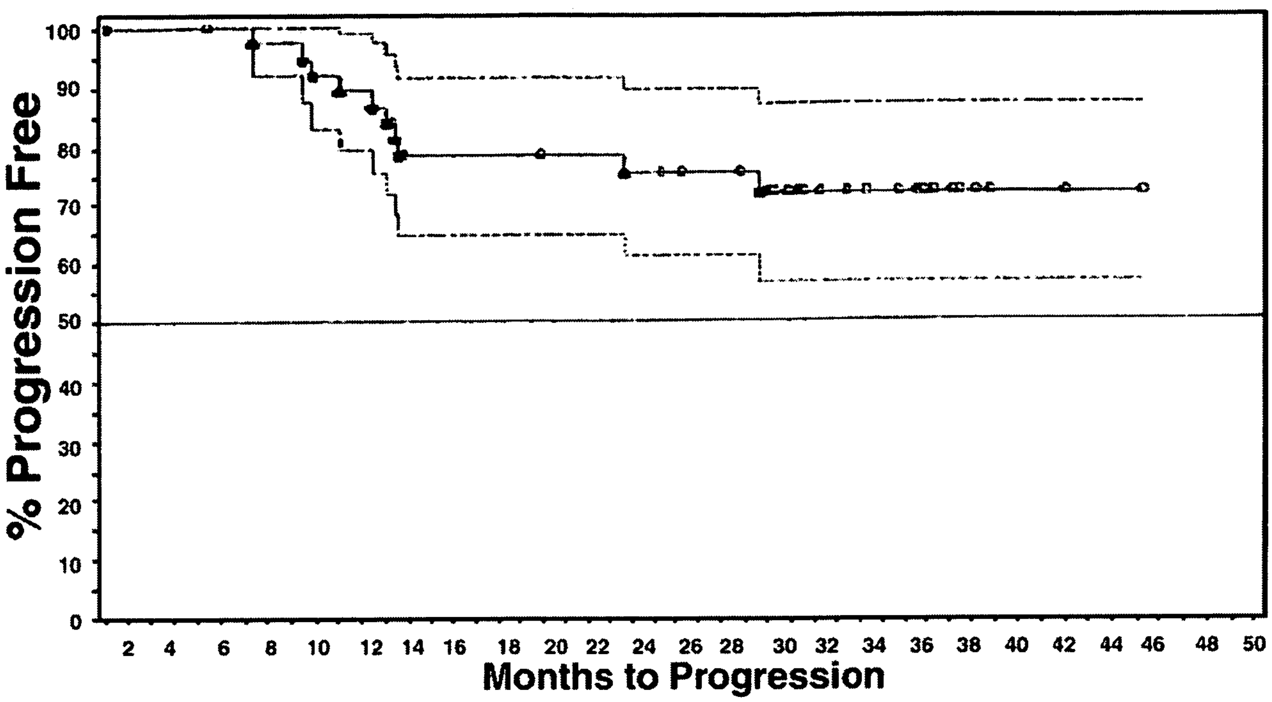

Fig 2. Kaplan-Meier analysis of time to progression, performed in 38 assessable patients.

with no difference in survival as compared with other treatment modalities.[17] The CHOP chemotherapy regimen has been studied extensively in NHL, and high response rates have been reported.[18-24] In 1976, the Southwest Oncology Group[18] (SWOG) reported on 204 stage III and IV assessable patients treated with CHOP chemotherapy that was repeated every 2 to 3 weeks (for six or more cycles), followed by maintenance with cyclophosphamide, vincristine, prednisone or vincristine, cytarabine, prednisone every 3 to 4 weeks for a total of 18 months in patients achieving CR status. A 78% complete response rate was reported in the subpopulation of 73 patients with nodular lymphoma and a 61% response rate in the subpopulation of 23 patients with well-differentiated lymphocytic lymphoma.

Table 4. Tumor Response and Duration in Nine Previously Treated Patients: Last Chemotherapy Versus CHOP + Rituxan

	Last Prior Chemotherapy		CHOP + Rituxan	
Patient No.	Response	Response Duration* (months)	Response	Response Duration* (months)
2	PR	4	PR	5.1
6	CR	5	CR	10.2
13	PR	28	CR	35.7+
17	PD	0	CR	33.3+
19	CR	15	PR	4.1
22	CR	24	PR	10.5+
27	CR	12	CR	26.3+
28	CR	50	CR	28.5+
29†	—	—	—	—

*Median observation time, 29+ months.

†Patient withdrew from study before initiation of CHOP + Rituxan treatment, and prior response data were not available.

Jones et al[19] reviewed the SWOG experience with CHOP from 1997 to 1983 in patients with no prior chemotherapy, advanced stage (III or IV), or recurrence after chemotherapy (any stage). Treatment was administered every 3 weeks for eight cycles. In 281 patients with favorable histology, the CR rate was 57% to 64%, without significant differences between CHOP or CHOP plus levamisole with or without BCG vaccine. Subsequently, Dana et al[20] reviewed these two SWOG studies, as well as an additional SWOG lymphoma study, and identified 415 stage III or IV low-grade lymphoma patients who had no prior therapy and were treated with full-dose CHOP. A CR rate of 64% and a median survival duration of 6.9 years was observed in these patients. Overall, chemotherapy or levamisole-BCG maintenance treatment did not increase the overall survival of patients achieving a CR with CHOP.

McLaughlin et al[22] added bleomycin and radiotherapy to the CHOP regimen for treatment of 74 patients with stage III follicular lymphoma. Chemotherapy was administered every 3 weeks for 10 cycles, with radiotherapy given after two cycles of chemotherapy. All patients were previously untreated. These investigators reported a 97% CR rate for

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Table 5. All Adverse Events by Grade (Incidence by Patient*) (N = 40)

	Grade				Total	
	1	2	3	4	No.	%
Any adverse event	1	7	12	18	38	95.0
Neutropenia	2	5	9	15	31	77.5
Alopecia	7	16	6	1	30	75.0
Nausea	20	6	1	0	27	67.5
Fever	5	16	2	0	23	57.5
Leukopenia	5	4	6	4	19	47.5
Anemia	0	14	2	0	16	40.0
Asthenia	12	3	1	0	16	40.0
Vomiting	6	8	1	0	15	37.5
Infection	8	5	1	0	14	35.5
Headache	8	5	0	0	13	32.5
Anxiety	8	3	0	0	11	27.5
Chills	6	4	1	0	11	27.5
Constipation	7	4	0	0	11	27.5
Pain	7	3	0	0	10	25.0
Pharyngitis	9	1	0	0	10	25.0
Rhinitis	9	1	0	0	10	25.0
Cough increase	7	2	0	0	9	22.0
Dyspepsia	6	3	0	0	9	22.0
Myalgia	6	3	0	0	9	22.0
Stomatitis	3	5	1	0	9	22.0
Arthralgia	6	1	1	0	8	20.0
Dyspnea	4	3	1	0	8	20.0
Insomnia	7	1	0	0	8	20.0
Thrombocytopenia	3	1	2	2	8	20.0
Diarrhea	5	1	0	0	6	15.0
Esophagitis	3	2	1	0	6	15.0
Pruritus	5	1	0	0	6	15.0
Sinusitis	4	2	0	0	6	15.0
Dizziness	4	1	0	0	5	12.5
Hyperglycemia	1	2	1	1	5	12.5
Hypertonia	4	1	0	0	5	12.5
Abdominal pain	4	0	1	0	5	12.5
Paresthesia	5	0	0	0	5	12.5
Weight decrease	3	2	0	0	5	12.5

*At least 5% of patients and any grade 3 or 4 event.

follicular small-cleaved NHL, a 73% CR rate for follicular-mixed NHL, and a 57% CR rate for patients with follicular large-cell NHL. The overall survival rate was 71% at 5 years and 56% at 7 years; 5-year relapse-free survival was 52%. Survival was influenced by histology, with follicular small-cleaved cell (n = 38) and follicular-mixed (n = 15) (91% and 84% survival at 5 years, respectively) more successful than follicular large-cell (n = 21) (40% survival at 5 years).

It should be noted that many of these early published CHOP response data were determined at a time when computed tomographic scanning was not available and/or a variety of restaging studies were performed that were less stringent than the ones used in the current trial. In a more recent study,[25] in which 83 patients with previously untreated follicular lymphoma (IWF types B and C) received six to eight cycles of CHOP chemotherapy, staging studies included computed tomographic scans, flow cytometric analysis of peripheral blood and bone marrow samples in every patient, and gallium scanning as needed to determine the extent of disease. In contrast to the high response rates reported in earlier studies, only 28 patients (36%; 90% confidence interval, 27% to 46%) achieved a CR[25] with more stringent clinical staging evaluations. The 55% CR rate in the 40 intent-to-treat patients (including nine patients with prior chemotherapy) on our current study compares favorably with this more recent CHOP study and strongly suggests that Rituxan contributes additional antitumor activity to patients treated with six cycles of CHOP chemotherapy, as evaluated by similar staging studies.

The toxicity of CHOP chemotherapy has been described in studies of low-, intermediate-, and high-grade lymphoma. In a randomized study comparing CHOP with other aggressive regimens, 1% of patients experienced fatal toxicity in the CHOP group and an additional 31% developed grade 4 life-threatening toxicities.[26] SWOG[19] reported fatal toxicities in 3% of patients and grade 3 and 4 toxicities in an additional 28% of patients. Hematologic toxicity with secondary infection and anemia were the most prominent adverse effects. In one study, six of 20 patients (30%) developed pancytopenia (absolute granulocyte count < 500/mm^3 and platelets < 50,000/mm^3). The Eastern Cooperative Oncology Group (ECOG) reported that 17 of 19 patients (89%) developed grade 3 and 4 leukopenia, two of 19 patients (11%) had grade 3 thrombocytopenia, and 14 of 19 patients (74%) had grade 3 anemia. Sixty-three percent of patients who received CHOP required at least one admission to the hospital. Most hospitalizations were due to neutropenic fevers (nine of 19 patients [47%] had a total of 13 admissions).[27] In another study, six of 20 patients developed documented infections.[28] Furthermore, reactivation of hepatitis B has been attributed to immunosuppression secondary to cytotoxic drugs, corticosteroids, etc, in other studies.[29-31] Grade 1 neurologic disorders[32] developed in 59% of patients in one study, and ECOG reported that two (11%) of 19 patients had developed grade 3 neurologic adverse events related to vincristine. Sixty percent of patients developed grade 1 and another 30% developed grade 2 gastrointestinal toxicity. One hundred percent of patients developed alopecia. Forty percent of patients developed a decrease in left ventricular ejection fraction, and 20% experienced cardiac arrhythmias. One of 20 patients developed congestive heart failure after a cumulative doxorubicin dose of 294 mg/m^2. CHOP has also been associated with anxiety, rash, and decreased sexual interest.[33]

In the present study of 31 patients with untreated NHL and nine patients with relapsed low-grade or follicular NHL, the combination of Rituxan and CHOP chemotherapy yielded an overall response rate of 95% (38 of 40 patients) in the intent-to-treat population, with a median time to progression

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that had not yet been reached at a median observation time of 29+ months. Patients with poor prognostic factors, such as extranodal disease, elevated serum lactate dehydrogenase levels, bone marrow involvement, advanced age, and bulky disease, responded to the combination of Rituxan and CHOP.

The *bcl*-2 proto-oncogene is associated with a t(14;18) chromosomal translocation and has been reported in approximately 80% of low-grade NHL and 30% of intermediate-grade NHL.[3] The resultant *bcl-2* activation leads to overexpression of *bcl-2* protein, which inhibits apoptosis and is believed to play an important role in lymphomagenesis. Gribben et al[10,34] have previously demonstrated that the presence of *bcl-2* translocation-positive cells in marrow after autologous bone marrow transplantation, as measured by a sensitive PCR assay, has prognostic value in predicting relapse. In the CHOP-Rituxan study, serial *bcl-2* analysis was not planned by protocol but was evaluated prospectively at one center (Roswell Park Cancer Institute) using essentially the same PCR assay as that used by Gribben et al. Eight of 18 tested patients were found to be *bcl-2* positive in blood and/or marrow pretreatment. Seven of eight patients converted to PCR negativity after the completion of therapy. Six of these patients underwent elective autologous bone marrow collection posttreatment, with pooled buffy coat specimens confirming PCR negativity in all six patients. Unpurged stem cells were cryopreserved and are available for future autologous bone marrow transplantation if needed in those patients requiring dose-intensive therapy of refractory disease. The seven patients becoming *bcl-2* negative also had documented complete remissions by standard restaging evaluations and were considered to have achieved molecular complete remissions. Six patients remained in CR, and five of seven patients remained PCR negative by serial analysis for at least 24 months or longer. Standard-dose CHOP alone is incapable of converting *bcl-2*–positive bone marrow to PCR negativity.[35] The potential impact of achieving and maintaining a posttreatment molecular CR with respect to disease-free and overall survival in *bcl-2*–positive NHL patients in a non–autologous bone marrow transplantation setting will be studied prospectively in large cohorts of patients in future Rituxan trials.

These clinical findings suggest that Rituxan adds therapeutic benefit to CHOP therapy without causing significant additional toxicity. Owing to the promising results from this chemoimmunotherapy trial, a multicenter study of this combination in previously untreated intermediate- and high-grade NHL patients has recently been completed and a single institution study in previously untreated mantle-cell lymphoma patients is ongoing at Dana-Farber Cancer Institute. Many other clinical trials for the study of Rituxan in combination with a variety of other cytotoxic agents for the treatment of CD20-positive neoplasms in a variety of clinical settings are being planned for the future.

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Current therapeutic paradigm for the treatment of non-Hodgkin's lymphoma.

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Patients with indolent non-Hodgkin's lymphoma may be treated with various approaches ranging from deferred initial therapy (watch and wait) to single-agent alkylating agents, radiation therapy, or combination chemotherapy. None of these approaches have produced curative results. Clearly, innovative treatment strategies are needed. The use of interferon, monoclonal antibodies with or without radioisotopes, purine analogues, and even high-dose therapy with stem-cell rescue are under investigation. Based on the fact that fewer than 40% of advanced-stage, aggressive non-Hodgkin's lymphoma patients are cured with cyclophosphamide/doxorubicin/vincristine/prednisone chemotherapy, the best approach for any patient is an experimental one. Examples include: (1) increasing the dose intensity of drugs used in standard regimens; (2) preventing the development of drug resistance; (3) combining monoclonal antibodies with chemotherapy; or (4) autologous stem-cell transplantation as a rescue from marrow-ablative chemotherapy. If a patient is not eligible or does not wish to participate in a clinical trial, cyclophosphamide/doxorubicin/vincristine/prednisone, as inadequate as it is, remains the gold standard.

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What Is New in Lymphoma?

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The article is available online at http://CAonline.AmCancerSoc.org

ABSTRACT The lymphomas are a diverse group of malignant disorders that vary with respect to their molecular features, genetics, clinical presentation, treatment approaches, and outcome. Over the past few years, there have been major advances in our understanding of the biology of these diseases, leading to a universally adopted World Health Organization classification system. New therapies are now available with the potential to improve patient outcome, and the International Prognostic Index and standardized response criteria help make clinical trials interpretable. Most notably, the chimeric antiCD20 monoclonal antibody rituximab has altered our therapeutic paradigms for B-cell disorders. Combinations of this antibody with chemotherapy and other biologic agents have shown promise in treating lymphoma. Other antibodies, radioimmunoconjugates (such as Y-90 ibritumomab tiuxetan and I-131 tositumomab), and oblimerson sodium (a *BCL-2* antisense oligonucleotide) have all shown promise. New chemotherapy regimens such as bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP), agents such as gemcitabine, and monoclonal antibodies directed against CD30 are also being studied in Hodgkin Lymphoma. The challenge of clinical research is to optimize the use of these agents, select patients most likely to respond, and develop multitargeted strategies based on sound scientific rational, with the potential to increase the cure rate of patients with lymphomas. *(CA Cancer J Clin 2004;54:260–272.)* © *American Cancer Society, Inc., 2004.*

Lymphomas represent about 4% of the new cases of cancer diagnosed in the United States each year, making them the fifth most common cancer diagnosis and the fifth leading cause of cancer death. An estimated 62,250 people will be diagnosed with lymphoma in 2004, of which 54,370 are non-Hodgkin Lymphomas (NHLs), with the remainder being Hodgkin Lymphoma (HL).[1] In fact, while the incidence of most cancers is decreasing, lymphoma is one of only two tumors increasing in frequency, although the cause for this increase is unknown.

NON-HODGKIN LYMPHOMA

Classification

The NHLs represent a clinically diverse group of diseases of either B-cell or T-cell origin. For several decades, they were classified according to the International Working Formulation which was primarily based on morphologic appearance and, to a lesser extent, clinical behavior. In 1994, the Revised European-American Lymphoma (REAL) Classification distinguished lymphomas not only by histology, but by immunophenotypic, genetic, and clinical characteristics.[2] This system was further modified as the now universally accepted World Health Organization (WHO) classification (Tables 1, 2).[3]

Prognosis

In general, the NHLs are divided into diseases that are indolent, aggressive, and very aggressive. However, even within histologic subtypes, patients vary considerably with regard to outcome. In 1993, the International Prognostic Index (IPI) was published, which was developed using data from a large number of similarly treated patients with diffuse large B-cell NHL.[4] Based on age, performance status, serum lactate dehydrogenase (LDH), number of

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TABLE 1 World Health Organization Classification of B-Lymphoid Neoplasms*

Precursor B-cell neoplasms
Precursor B-lymphoblastic leukemia/lymphoma
Mature (peripheral) B-cell neoplasms
B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Splenic marginal zone B-cell lymphoma
Hairy cell leukemia
Plasma cell myeloma/plasmacytoma
Extranodal marginal zone B-cell lymphoma of MALT type
Nodal marginal zone B-cell lymphoma
Follicular lymphoma
Mantle-cell lymphoma
Diffuse large B-cell lymphoma
Burkitt's lymphoma

*Common entities are shown in boldface type.

TABLE 2 World Health Organization Classification of T- and NK-Lymphoid Neoplasms*

Precursor T-cell neoplasm
Precursor T-lymphoblastic lymphoma/leukemia
Mature (peripheral) T-cell neoplasms
T-cell prolymphocytic leukemia
T-cell granular lymphocytic leukemia
Aggressive NK-cell leukemia
Adult T-cell lymphoma/leukemia
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic gamma-delta T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides/Sezary syndrome
Peripheral T-cell lymphoma, not otherwise characterized
Angioimmunoblastic T-cell lymphoma
Anaplastic large-cell lymphoma

*Common entities are shown in boldface type.

extranodal sites of involvement, and stage, patients could be separated into clinically distinct groups. More recently, a similar system has been developed for follicular lymphomas, referred to as the Follicular Lymphoma IPI (FLIPI).[5]

Newer technologies, such as DNA microarray analyses that identify genes that are either overexpressed or underexpressed by the malignant cells, have further distinguished patients into distinct risk groups even within histology.[6,7] For example, there appear to be at least two subcategories of diffuse large B-cell NHL, a germinal center B-cell type and a less favorable activated B-cell type that behave very differently, even within IPI categories.

Advances in the Treatment of Follicular NHL

The follicular lymphomas are the most common subtype of indolent NHL, representing about 30% of NHLs. They are characterized by an indolent clinical course with a median survival of 6 to 10 years. Only about 10 to 15% of patients present with limited (Stage I or nonbulky Stage II) disease. For those patients, radiation therapy may result in prolonged disease-free survival. Whether or not they are cured is controversial since relapses occur even after 10 to 20 years.

Until recently, no particular treatment clearly prolonged the survival of patients with advanced stage follicular NHL. As a result, a watch-and-wait approach was routinely recommended until treatment was clinically indicated because of disease-related symptoms, massive lymphadenopathy or hepatosplenomegaly, potential organ compromise, or peripheral blood cytopenias related to bone marrow involvement.

Despite decades of clinical research, there is still no consensus as to the optimal initial therapy for follicular and low-grade NHL. Neither an alkylating agent alone or combined with vincristine and prednisone (eg, CVP), CVP with doxorubicin (CHOP), nor fludarabine-based regimens produce a clear survival advantage over any other.

Rituximab

The availability of active monoclonal antibodies has revolutionized our approach to indolent B-cell malignancies (Table 3). Rituximab, a chimeric anti-CD20 monoclonal antibody was originally studied in patients with relapsed and refractory follicular and low-grade NHL. In the pivotal trial of 166 patients, a dose of 375 mg/m^2 weekly for four weeks was associated with responses in almost half of patients (including 6% complete remissions) with a duration of response of about 11 months.[8] This antibody has been widely adopted because of its activity and favorable toxicity profile. Most adverse reactions occur during the infusion and consist primarily of fever and chills, with occasional hypotension.

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TABLE 3 Monoclonal Antibodies/RICs for non-Hodgkin Lymphoma

Antibody	Antigen	Conjugate
Rituximab	CD20	None
CAMPATH-1H	CD52	None
Epratuzumab	CD22	None, I-131, Y-90
Apolizumab (Hu1D10)	HLA-DR	None
Galiximab	CD80	None
Humanized CD20	CD20	None
Bevacizumab	VEG-F	None
Tositumomab (Bexxar)	CD20	I-131
Ibritumomab (Zevalin)	CD20	Y-90

Recent clinical trials have attempted to improve on the activity of rituximab. Increasing the number of weekly infusions from four to eight, delivery of higher doses, and increased dose density have all been unsuccessful in this regard.[9–11] Higher overall response rates have been observed with rituximab as initial therapy;[12,13] however, the duration of response has been disappointing.

The possible benefit of maintenance therapy has also been evaluated in an attempt at prolonging the time to disease progression. Hainsworth et al.[13] treated 62 patients with follicular and low grade NHL using four weekly doses of rituximab followed by four additional doses every six months for two years. The time to progression of 32 months was longer than expected. In a randomized trial, Ghielmini et al.[14] reported previously treated (n = 128) and previously untreated patients (n = 57) who received four weekly doses of rituximab, followed by a randomization to no further therapy or maintenance consisting of a single infusion of rituximab every two months for a total of eight months. The time to progression was significantly longer in the group that received maintenance; however, this benefit was primarily restricted to the previously untreated group.

The role of maintenance is confounded by the observation that 40% of patients who have experienced an initial response lasting at least six months will respond a second time, with a duration of response at least as long as the initial response.[15] Therefore, an important question is whether it is preferable to deliver maintenance or to treat disease progression instead. Hainsworth et al.[16] attempted to answer this issue in a study in which patients who were treated with an initial four weeks of rituximab were then randomized to maintenance therapy, as previously published,[13] or to retreatment on recurrence. Although response rates and time to progression favored the maintenance arm, the time to which another treatment other than rituximab was required was similar (31 months versus 27 months). The Eastern Cooperative Oncology Group (ECOG) rituximab extended schedule or retreatment trial is comparing treatment until relapse with retreatment at the time of recurrence. Therefore, at the present time, the preferable approach is not clear.

In vitro studies suggest that monoclonal antibodies such as rituximab can sensitize lymphoma cells to the effects of subsequent chemotherapy.[17,18] These observations have been supported by numerous reports in which results with rituximab plus chemotherapy appear superior to what would be expected with chemotherapy alone. The first of these regimens was reported by Czuczman et al.[19] in which 38 patients, 31 of whom were previously untreated, received CHOP plus rituximab. The overall response rate was 100%, with 58% complete remissions and a median time to progression of 8.3 years.[20] Comparable response rates can be achieved with a variety of other rituximab-based chemotherapy regimens[21–29] (Table 3). For example, Czuczman et al.[21] have also reported on the combination of fludarabine plus rituximab with a response rate of 93% and 80% complete responses. However, any differences in complete or overall response rates among the various regimens may be related to patient selection or the point in time when response is assessed, as maximal responses may occur several months following therapy.

Recent randomized trials have shown superiority for rituximab-containing regimens over chemotherapy alone. The German Low Grade Lymphoma Study Group conducted a randomized study of 394 patients who were allocated to either CHOP or R-CHOP, with a secondary randomization to a variety of postremission therapies.[28] There was no advantage from rituximab in response rate (97% versus 93%), but the combination was associated with longer

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event-free survival and a trend toward longer overall survival. The variety of postremission therapies makes these data difficult to interpret.

Marcus and coworkers[27] reported on 322 patients with either intermediate or poor risk follicular NHL who received CVP either alone or with rituximab (R-CVP). The overall response rate and complete response rates for the combined modality therapy were 81% and 40%, respectively, versus 57% and 10% for CVP alone. With a median follow-up of 18 months, the R-CVP patients had a significantly longer median time to treatment failure of 27 months, versus 7 months for CVP. In addition, the median time to treatment progression was not reached for R-CVP, compared with 113 months for CVP alone. Whether an eventual prolongation in survival will be achieved with any of these regimens remains to be demonstrated by longer follow up. Thus, a clinical trial remains the preferred option for the initial therapy for patients with follicular or low-grade NHL. In the future, the optimal treatment may be determined by clinical and biological characteristics of individual patients.

However, it is clear that not all patients respond to rituximab nor benefit from the addition of that antibody to chemotherapy regimens. Patients most likely to respond can be predicted by polymorphisms for FcR gamma III, which represent the binding site for the rituximab antibody,[30] and DNA microarray signatures.[31] Moreover, the benefit of rituximab appears to be limited to patients whose tumors overexpress the *BCL-2* gene.[32] Whether these observations will determine which patients will receive rituximab remains to be seen.

Other Monoclonal Antibodies

Other unconjugated antibodies being evaluated include epratuzumab, apolizumab, alemtuzmab, galiximab, and several humanized anti-CD20 antibodies; however, a major role for any of these in NHL is uncertain at present. Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against the CD52 antigen, whose exact function remains unknown; it is expressed on the surface of all lymphocytes, monocytes, macrophages and eosinophils. Although alemtuzumab appears to be very active in chronic lymphocytic leukemia as well as T-cell lymphomas, its activity in B-cell NHL is disappointing with partial responses of 14%.[33,34]

Epratuzumab is a humanized IgG1 monoclonal antibody directed against the CD22 antigen, expressed in a variety of lymphomas. In a dose escalation study of epratuzumab in 55 patients with indolent NHL, no dose limiting toxicities were identified. The overall response rate was 24% in patients with follicular histologies.[35] However, results from a subsequent trial designed to evaluate the combination of rituximab and epratuzumab was disappointing, suggesting that a suboptimal dose and schedule of these agents was selected.[36]

Apolizumab (Hu1D10, Remitogen) is a humanized monoclonal antibody directed against a polymorphic determinant of HLA-DR, found on both normal B cells and in about half of patients with lymphoid malignancies. Although Phase I data revealed activity in patients with relapsed or refractory indolent NHL, the Phase II data were disappointing. Furthermore, toxicities including thromboses and hemolytic uremic syndrome have hindered its development.[37–39]

CD80 is an immune costimulatory molecule present on the surface of NHL cells. Galiximab is a macaque-human chimeric anti-CD80 antibody with in vivo antilymphoma properties, which is actively being studied in patients with refractory NHL. The antibody is well tolerated except for mild fatigue, nausea, and headaches, and has single agent activity of about 15%.[40] Based on preclinical data suggesting synergy, a Phase I/II study of the combination of galiximab and rituximab has recently been completed and is undergoing analysis.[41]

Radioimmunotherapy

Despite the encouraging results with rituximab, all patients eventually become resistant to this agent. A number of reasons have been proposed including inadequate serum concentrations, loss of expression of CD20, and inaccessibility of the tumor cells to the antibody. One attempt to overcome these problems is the use of radioimmunotherapy (RIT), in which a

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monoclonal antibody is conjugated to a radioisotope. RIT kills not only cells to which the antibody is bound, but as a result of a cross fire effect, also kills neighboring cells that may not express the antigen or which are inaccessible to the monoclonal antibody.

Two radioimmunoconjugates are currently commercially available. Y-90 ibritumomab tiuxetan (Zevalin) is a murine rituximab conjugated to Y-90.[42] The Zevalin regimen takes about eight days to administer. On the first day, a dose of cold (nonradioactive) anti-CD20 antibody is administered to bind nontumor CD20 sites and to facilitate better biodistribution. Because Y-90 is a beta emitter, it cannot be used for imaging; thus, indium-111 labeled ibritumomab is substituted. Two to three sets of imaging studies are performed at days 0, 2 to 3, and 6 to 7 to ensure appropriate biodistribution. On day 7 to 8, another dose of cold antibody is delivered, followed by 0.4 mCi/kg of Zevalin (not to exceed 32 mCi) for patients with platelet counts of at least 150,000/mm^3. The dose is reduced to 0.3 mCi in patients with a platelet count of 100 to 149,000/mm^3. The clinical trials thus far conducted with radioimmunoconjugates have demonstrated that they are active (more active than their cold antibody) and are useful in patients who have relapsed after, or who are refractory to, rituximab. The response rate with Zevalin in rituximab failures is reported to be 74% with 15% complete remissions.[43] In a randomized trial, 143 patients with relapsed CD20 positive NHL, without previous rituximab exposure, received either rituximab or Zevalin. Response rates were higher in the Zevalin arm at 80% compared with 56% in the rituximab arm,[44] supporting the additive benefit of the radioisotope. However, there was no difference between the arms in time to disease progression. Responses can also be safely achieved in patients who have mild thrombocytopenia (100 to 149,000/mm^3) using a lower dose of Zevalin.[45]

The major complications following Zevalin therapy relate to its myelosuppression which occurs later than with chemotherapy, at around 7 to 9 weeks following treatment. As a result, exclusionary criteria for the use of Zevalin therapy include greater than 25% bone marrow involvement, a hypocellular bone marrow (<15% cellular), platelets <100,000/mm^3, neutrophils <1,500/mm^3, extensive prior radiation therapy, and prior stem cell transplantation—the latter because the safety in this setting is unknown.

Bexxar is a conjugate of the murine anti-CD20 antibody tositumomab and I-131. Since I-131 is a gamma emitter, dosimetry can be performed to provide patient-specific dosing. As with Zevalin, treatment occurs over about a week. In contrast, thyroid protection is required with Bexxar because of the radioactive iodine. In a multicenter pivotal trial,[46] 60 heavily pretreated patients with NHL received Bexxar, with response rates of 65% with 20% complete response for the group as a whole. Patients with follicular histologies fared better, with an overall response rate of 81%. Response rates and response duration were significantly higher than with their last chemotherapy. In rituximab refractory patients, the response rate has been 63% with 29% complete response. Bexxar was subsequently approved for use in patients with relapsed/refractory follicular or transformed NHL. It has also been safely administered to previously untreated patients, with a response rate of 97% and 63% complete responses.[47] The major toxicity is myelosuppression. Bexxar can be safely administered after CHOP chemotherapy, and converts some partial responses to complete responses.[48] A randomized Phase III of a CHOP followed by Bexxar regimen versus R-CHOP is ongoing within the Southwest Oncology Group (SWOG) and the Cancer and Leukemia Group B (CALGB).

One of the major concerns with RIT is the development of secondary acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS). Published data suggest that the risk is about 1.5% with Zevalin and 6.3% with Bexxar. However, the development of this secondary malignancy likely relates to the prior treatment and may not be greater than expected from chemotherapy alone.[46,49,50]

Preliminary reports with both Zevalin and Bexxar suggest that patients can tolerate additional therapies; however, the response and toxicity data are not yet available.[51,52]

RIT may also be useful in the stem cell transplant setting. Gopal and coworkers compared their results with follicular lymphoma patients

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who received high dose I-131 tositumomab with those treated using various high dose chemotherapy regimens. They found better overall survival and progression free survival, with lower toxicity in the RIT-treated population.[53]

Zevalin and Bexxar appear to have comparable activity, and their relative toxicity is being tested in a large Phase III trial. Current research is directed at trying to combine or sequence radioimmunotherapy with chemotherapy and other biologicals.

Other New Agents

Antisense Oligonucleotides

Antisense oligonucleotides are chemically modified single-strand DNA molecules that have a nucleotide sequence that is complementary to the target mRNA; therefore, they are capable of inhibiting expression of the target gene. The *BCL-2* gene is a potentially important target because it is overexpressed in most follicular B-cell NHLs and chronic lymphocytic leukemias, and in about a quarter of large B-cell NHL. *BCL-2* upregulation is thought to be responsible for maintaining the viability of tumor cells, as well as inducing a form of multidrug resistance. Elevated *BCL-2* also correlates with poor response to therapy in NHL. These observations, and others, have stimulated interest in exploring an antisense strategy against *BCL-2* and other genes important to tumor survival (Table 4).

To inhibit the target mRNA, antisense oligonucleotides must first be incorporated into cells by endocytosis. The oligonucleotide then inhibits gene expression by hybridization with the mRNA, followed by cleavage of the mRNA by recruitment of RNAse-H and other endonucleases.

G3,139 (oblimersen sodium; Genasense, Genta Incorporated, Berkeley Heights, NJ) is the first antisense molecule to be widely tested in the clinic for the treatment of human tumors. G3,139 is a phosphorothioate oligonucleotide consisting of 18 modified DNA bases (ie, 18-mer) that targets the first 6 codons of *BCL-2* mRNA to form a DNA/RNA duplex.

In the first Phase I study of G3,139 in 21 patients with NHL,[54] one patient with low-grade lymphoma who had progressive disease in nodes and bone marrow after two prior regimens attained a complete response, which has been maintained for longer than three years. Subjective improvement was also noted in the majority of patients who entered the study with tumor-related symptoms.

TABLE 4 Drugs Being Studied in non-Hodgkin Lymphoma

Drug	Mechanism of Action	Phase of Clinical Testing in Lymphoma
Bortezomib	Proteasome and NFκB inhibition	Phase II
Gallium nitrate	Inhibits ribonucleotide Reductase/DNA synthesis	Phase II
Bendamustine	Alkylating/antimetabolite	Phase II
Oblimerson sodium	*Bcl-2* antisense	Phase II
SAHA and Depsipeptide	Histone deacetylase inhibition	Phase II
CCI-779	Inhibits mTOR	Phase I/II

TABLE 5 World Health Organization Classification of Hodgkin Lymphoma*

Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
- **Nodular sclerosis Hodgkin lymphoma**
- Lymphocyte-rich classical Hodgkin lymphoma
- **Mixed cellularity Hodgkin lymphoma**
- Lymphocyte depletion Hodgkin lymphoma

*Common entities are shown in boldface type.

This *BCL-2* antisense is also active in relapsed/refractory patients with mantle cell lymphoma (MCL).[55] Side effects primarily include neutropenia, thrombocytopenia, and fatigue. Although the response rate to the single agent is modest, it augments the activity of other agents, such as rituximab, fludarabine and cyclophosphamide; therefore, this drug will have its greatest impact in combination strategies. Such multiagent regimens are currently under clinical investigation.

Vaccines

Lymphomas are characterized by their own unique idiotype, the variable region of the immunoglobulin light chain, providing the pos-

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sibility for a lymphoma vaccine. In a study from Stanford, 49% of follicular NHL patients treated with this protein, conjugated to an adjuvant such as keyhole limpet hemocyanin (KLH), reacted with a cellular and humoral immune response. The ability to generate such a response appears to correlate with time to tumor progression (7.9 years versus 1.3 years).[56] Three randomized studies are evaluating the clinical benefit of the antiidiotype vaccine. In two of these, patients receive chemotherapy, followed by a rest period, and subsequently a series of vaccinations with either antiidiotype vaccine plus GM-CSF and KLH, or GM-CSF and KLH alone. In the third trial, rituximab is used as initial treatment, followed by GM-CSF and KLH with or without the antiidiotype vaccine.

Advances in the Treatment of Aggressive NHL

For decades, CHOP remained the standard regimen for patients with diffuse large B-cell NHL. Using this relatively well-tolerated regimen, about 40% of patients were cured with prolonged follow up. More intensive and aggressive regimens failed to demonstrate an advantage in randomized trials. Rituximab as a single agent was shown to have a response rate of 33% leading to interest in combining this antibody with chemotherapy. In initial studies by Vose and coworkers, the complete and overall response rates to R-CHOP were higher than would be expected with CHOP alone.[57] A marked paradigm shift followed the 2002 publication by the Groupe d'Étude des Lymphomes Agressifs (GELA) group of their randomized trial in 399 patients between the ages of 60 to 80 years with diffuse large B-cell NHL (DLBCL),[58] who received either CHOP alone or R-CHOP given on day 1 of each cycle. The complete response rate (76% versus 63%), as well as event-free and overall survival, significantly favored the combination arm. Although the difference in event-free and overall persists, there is some convergence of the overall survival curves over time. An ECOG, CALGB, and SWOG intergroup study compared CHOP with R-CHOP in 632 patients with diffuse large B-cell NHL over the age of 60 years.[59] The study design also included a secondary randomization to rituximab maintenance or observation. In contrast to the previously published GELA study, there was no difference in response rates, time to treatment failure (TTF) or survival by induction regimen. An unplanned analysis performed to remove the confounding effect of rituximab maintenance suggested a benefit for R-CHOP with respect to TTF and survival noted only in patients who did not receive rituximab during induction. Thus, these studies support R-CHOP as the new standard for patients with diffuse large B-cell NHL.

Other Drugs fro Non-Hodgkin Lymphoma in Clinical Trials

Gallium Nitrate

Gallium nitrate, the salt of the element gallium, was one of the elements tested in the National Cancer Institute (NCI) screening system found to have anticancer activity. Human clinical trials were started in 1976; the drug was found to have a profound hypocalcemic effect and was approved by the Food and Drug Administration for the treatment of hypercalcemia. Gallium has been shown to be a targeted therapy as it localizes to tumor sites, and this finding has been exploited in gallium scans. Initial Phase I and II clinical trials in a variety of malignant lymphomas used brief infusions of the drug and were associated with excessive toxicity, including optic neuritis. As a result, lower doses of the drug were delivered by continuous infusion, with responses in 43% of patients with relapsed and refractory disease.[60] A multicenter Phase II confirmatory trial using contemporary diagnostic and response criteria has just been completed in the United States and is undergoing analysis.

Bendamustine

Bendamustine is a bifunctional compound with both an alkylating nitrogen mustard group and a purinelike benzimidazole ring. It was first synthesized in 1963 in the German Democratic

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Republic and has been used extensively in Germany since. Bendamustine has demonstrated activity in indolent and aggressive NHL, HL, chronic lymphocytic leukemia, and multiple myeloma.[61–64] In vitro and clinical data also support a beneficial interaction with rituximab. In a study of 63 patients with relapsed or refractory indolent NHL or mantle cell lymphoma (MCL), the response rate to this combination was 94% with 71% complete remissions.[29] This combination was extremely well tolerated. To better characterize the activity of this agent and to provide broader experience with the agent, it is now being studied alone and in combination with rituximab in Phase II trials in the United States.

Bortezomib

Bortezomib (PS-341; Velcade) is a potent, reversible inhibitor of the 26S proteasome, an enzyme important in the intracellular degradation of proteins including those involved in cell cycle regulation, transcription factor activation, apoptosis, and cell trafficking. Notable among these is NF-κB. Bortezomib is the first proteasome inhibitor to be clinically studied and has recently been approved by the Food and Drug Administration for the treatment of relapsed/refractory multiple myeloma.[65] The rationale for a study in NHL is that NF-κB is overexpressed in a number of histologies. In a report from Goy et al.[66] including 51 evaluable patients with a median of 3.5 prior treatment regimens (including eight patients with a prior autologous stem cell transplant), the response rate was 48% in those 23 evaluable patients with MCL, with 26% complete responses. A lower level of 16% was reported in small numbers of patients with other histologies. Toxicity included 22 patients with grade III thrombocytopenia, one patient with grade IV thrombocytopenia, and two patients with severe infections. A similar response rate in 10 patients with MCL has also been reported by O'Connor et al.,[67] who observed one complete and five partial responses in eight patients with follicular NHL, but failed to identify activity in three patients with small lymphocytic lymphoma. Preclinical models suggest that the activity of bortezomib is enhanced by *BCL-2* antisense, and this combination is being pursued in the clinic.

Histone Deacetylase Inhibitors

Depsipeptide (NSC 6,30176) is a bicyclic peptide originally isolated from *Chromobacterium violaceum*, strain 968, by Fujisawa Pharmaceuticals. Depsipeptide, either alone or in combination with hypomethylating agents, has been shown to induce a number of cellular proteins that may have critical effects on apoptosis, proliferation, and susceptibility to immunologic manipulation. The compound may also have antiangiogenic activity that contributes to antitumor efficacy. In a Phase I trial at the NCI, objective responses were reported in eleven patients; one complete response was reported in a peripheral T-cell lymphoma (PTCL) patient at the 12.7 mg/m^2 dose level.[68] In addition, partial responses were reported in nine cutaneous T-cell lymphoma (CTCL) patients at the 17.8 mg/m^2 dose level. Toxicities attributed to depsipeptide included anemia, leukopenia, neutropenia, thrombocytopenia, fatigue, anorexia, nausea, vomiting, elevated alanine aminotransferase/aspartate aminotransferase, increased creatine phosphokinase (CPK), hypocalcemia, asymptomatic EKG changes (ST-T wave flattening and inverted T waves), and supraventricular arrhythmias (SVT/atrial fibrillation/flutter). A Phase II trial at the NCI is ongoing.

Another histone deacetylase inhibitor, suberoylanilide hydroxamiic acid (SAHA), has demonstrated activity in NHL in Phase I trials and is now undergoing Phase II testing.[69]

Rapamycin Analog

The macrolide rapamycin (sirolimus, Rapamuner, Wyeth-Ayerst, Princeton, NJ) and its derivatives inhibit the mammalian target of rapamycin (mTOR), downregulating translation of specific mRNAs required for cell cycle progression from G1 to S phase.[70] Preclinically, mTOR inhibitors potently suppress growth and proliferation of lymphocytes and tumor cell lines.[70] Today, rapamycin is approved as an immunosuppressive drug for renal transplant

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recipients. A related compound, CCI-779 (Wyeth-Ayerst, Princeton NJ) is being developed as a cancer therapeutic. In early studies, activity has been seen in mantle cell lymphoma, but with considerable myelotoxicity.[40] Studies are being designed to explore this agent at more tolerable doses.

HODGKIN LYMPHOMA

HL accounts for 14% of lymphomas with an estimated 7,880 new cases in 2004.[1] Although the etiology of HL is not known, people with a history of infectious mononucleosis have a three-fold increased likelihood of developing HL, supporting a role for the Epstein-Barr virus.[71]

Classification

The WHO classification of 1999 recommended changing the name to Hodgkin Lymphoma and proposed two categories: classical HL[72] and nodular lymphocyte predominant HL. Classical HL is subdivided into nodular sclerosis (NS) HL, lymphocyte-rich classical (LRC) HL, mixed cellularity (MC) HL, and lymphocyte depletion (LD) HL. Nodular lymphocyte predominant HL is a unique form of HL that accounts for only 3 to 8% of cases of HL and generally exhibits a nodular growth pattern, with or without diffuse areas, and with rare Reed-Sternberg cells. The atypical lymphocytic and histiocytic (L&H) cells express B-cell antigens such as CD20, but rarely express CD15 or CD30, which are usually found in Classical HL. LPHL is more often localized than disseminated at diagnosis (>70% Stages I or II), exhibits a slowly progressive course, and has an extremely favorable outcome. Mediastinal masses are noted in fewer than 20% of cases. Although survival tends to be long, late relapses are more common than in other histologies, and 35% progress to a large B-cell NHL. Recent reports suggest activity for rituximab in patients with relapsed NLPHL,[73] with response rates up to 86% in one series, half of which are complete and many appear to be durable. However, while investigators from Stanford reported a high response rate of 100% with 46% complete remissions in previously treated[10] and untreated patients,[12] at a median follow-up of 13 months, 9 of the 22 patients had already relapsed.[74]

Treatment

Early Stage Disease

Radiation therapy (RT) has been the standard approach for patients with nonbulky Stage IA/IIA disease. RT achieves complete remissions in more than 95% of patients with limited disease, and the failure-free survival and overall survival rates are 75% and greater than 90%, respectively, beyond 20 years. However, radiation is associated with a number of unwanted effects, including an increased incidence of secondary malignancies. Therefore, goals of recent studies have been to determine the lowest effective dose of RT. In low risk patients, involved field irradiation has been shown to be comparable to total lymphoid irradiation. Current trials are seeking to determine whether chemotherapy can replace RT altogether. A recent randomized study compared six cycles of doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) plus involved field with four cycles and radiation, with a similar outcome. In another study, patients were treated with either four to six cycles of ABVD or standard therapy (subtotal nodal irradiation, with or without two cycles). The standard therapy produced a small but significant increase in time to progression, but with no difference in overall survival at the present time.[75]

Advanced Disease

Patients with advanced stage disease are those with Stages III or IV, the presence of B symptoms, and/or bulky disease (>10 cm at any site, >1/3 thoracic diameter). ABVD has become the standard chemotherapy because of a number of advantages; it is all-intravenous (providing better compliance), has less cumulative myelotoxicity, a lower risk of secondary malignancies (AML or solid tumors), and a lower rate of infertility compared with previous regimens (eg, mechlorethamine, vincristine,

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procarbazine, and prednisone). This regimen can induce complete remissions in 80 to 85% of patients, with five-year freedom from progression of 61% and an overall five-year survival of 73%.[76]

New Treatments for Hodgkin Lymphoma

The German Hodgkin Lymphoma Group developed the intensive bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbozine, and prednisone (BEACOPP) regimen to improve on the outcome of patients with high-risk disease. Escalated BEACOPP improved failure-free survival compared with cyclophosphamide, vincristine, procarbazine, and prednisone/ABVD, but with an increased risk of secondary AML/MDS.[77] The rate of progression during treatment was only 2%, with a relapse rate of 5%, a time to treatment failure of 88% at three years and overall survival of almost 95% at five years. Current studies are looking at a regimen of four cycles of escalated BEACOPP followed by four cycles of standard BEACOPP in an attempt at reducing toxicities. Another European trial is comparing escalated BEACOPP with ABVD.

Gemcitabine

Gemcitabine is a deoxycitidine analog with single agent activity in HL. When tested in a multicenter study in 23 patients with relapsed or refractory HL, excluding patients who had undergone autologous stem-cell transplantation, the toxicity was found to be manageable, with 9% complete response and 30% partial response.[78]Pulmonary toxicity of gemcitabine is uncommon and usually mild when given as a single agent, but can be unacceptably severe when used in combinations, such as being substituted for dacarbazine in ABVD or for etoposide in BEACOPP.

Nevertheless, other combinations including gemcitabine have been tolerable with high response rates, such as the CALGB regimen of gemcitabine, navelbine, and doxil initially studied in relapsed and refractory patients. An upfront trial of doxorubicin, vinblastine, and gemcitabine in the initial treatment of low-risk patients is now active.

Anti-CD30 Monoclonal Antibodies

The CD30 antigen expressed on both Reed-Sternberg cells in HL and the malignant cells of anaplastic large cell NHL provides an excellent target for antibody therapy. Several anti-CD30 antibodies are currently being studied in clinical trials. They have been well tolerated, but the dose and schedule need to be optimized for greater activity.[79]

ASSESSMENT OF RESPONSE

Standardized guidelines for response assessment facilitate interpretation of data, comparisons of the results among various clinical trials, and identification of new agents with promising activity, and also provide a framework on which to evaluate new biologic and immunologic insights into the diseases being studied.

Some of the differences among response criteria may appear subtle but have enormous implications. For a patient to be considered as having a complete response, a protocol generally requires that all lymph nodes that were involved with NHL return to normal size. However, what is considered "normal" varies among studies. Before treatment, a normal node is 1.0 cm in diameter. Nevertheless, following treatment, nodes rarely shrink below 1.0 cm, not because they are necessarily involved with tumor, but as the result of the presence of necrosis or fibrosis. In a study using the database generated from the 166 patient rituximab pivotal trial, the complete response rate was calculated using a bidimensional normal node size of 2.0 × 2.0 cm, 1.5 × 1.5 cm, or 1.0 × 1.0 cm. Whereas the overall response rate did not change appreciably (approximately 48%), the complete response rate significantly decreased from 28% to 18% to 6%.[80]

Recently published standardized response criteria have now been incorporated into lymphoma studies and are being used by regulatory agencies to evaluate new agents.[81] Nevertheless, these recommendations were based pri-

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marily on anatomic findings, including physical examination and CT scans. More recent studies suggest that positron emission tomography (PET) scans may be a more accurate way to distinguish between fibrosis and residual tumor posttreatment. In addition, whether a PET scan is positive or negative after one or two cycles of therapy is a strong predictor of outcome.[82–86] As a result, the current response criteria will be modified to incorporate PET scans.

FUTURE DIRECTIONS

This is an exciting time for the management of patients with lymphomas. Therapies are moving away from the nonspecific cytotoxic agents and toward more targeted approaches, especially in the NHLs where the malignant cells can be more reliably targeted. New classification schemes based on genetics and biology, and technologies such as genomics and proteomics, provide the opportunity to develop disease-specific and even patient-specific therapies. Not only have DNA microarrays identified different prognostic subsets, but also these subsets of patients may well be treated differently. Various approaches may be used to identify antibody-sensitive patients. At the same time, there is a growing list of new biologic and targeted chemotherapy agents. The challenge is to develop rational combinations and introduce these to patients who are most likely to benefit. The future lies in combining biologic therapies in a manner that will optimize their activity (with reduced dependence on the more toxic and nonspecific cytotoxic drugs), identifying those patients most likely to respond to those therapies, monitoring disease status, and preventing recurrence. Through these new technologies and with these unique agents, we will be able to improve the cure rate of patients with lymphomas.

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Merck Manual

Non-Hodgkin's lymphomas are a diverse group of cancers that develop in B or T lymphocytes.

This group of cancers is actually more than 20 different diseases, which have distinct appearances under the microscope, different cell patterns, and different clinical courses. Most non-Hodgkin's lymphomas (85%) are from B cells; less than 15% develop from T cells. Non-Hodgkin's lymphoma is more common than Hodgkin's disease. In the United States, about 65,000 new cases are diagnosed every year, and the number of new cases is increasing, especially among older people and people whose immune system is not functioning normally. Those at risk include people who have had organ transplants and some people who have been infected with the human immunodeficiency virus (HIV).

Although the cause of non-Hodgkin's lymphoma is not known, evidence strongly supports a role for viruses in some of the less common types of non-Hodgkin's lymphomas. A rare type of rapidly progressive non-Hodgkin's lymphoma, which occurs in southern Japan and the Caribbean, may result from infection with human T-cell lymphotropic virus type I (HTLV-I), a retrovirus similar to HIV. The Epstein-Barr virus is the cause of many cases of Burkitt's lymphoma, another type of non-Hodgkin's lymphoma.

Unusual Non-Hodgkin's Lymphomas

Mycosis fungoides is a rare, persistent, very slow-growing non-Hodgkin's lymphoma. Most people who develop it are older than 50. It originates from mature T lymphocytes and first affects the skin. Mycosis fungoides starts so subtly and grows so slowly that it may not be noticed initially. It causes a long-lasting, itchy rash—sometimes a small area of thickened, itchy skin that later develops nodules and slowly spreads. In some people, it develops into a form of leukemia (Sézary syndrome). In other people, it progresses to the lymph nodes and internal organs. Even with a biopsy, doctors have trouble diagnosing this disease in its early stages. However, later in the course of the disease, a biopsy shows lymphoma cells in the skin.

The thickened areas of skin are treated with a form of radiation called beta rays or with sunlight and corticosteroid drugs. Nitrogen mustard applied directly to the skin can help reduce the itching and size of the affected areas. Interferon drugs can also reduce symptoms. If the disease spreads to lymph nodes and other organs, chemotherapy may be needed. Without treatment, most people can expect to live 7 to 10 years after the diagnosis is made. Treatment does not cure the disease, but it slows it down even further.

Burkitt's lymphoma is a very fast-growing non-Hodgkin's lymphoma that originates from B lymphocytes. Burkitt's lymphoma can develop at any age, but it is most common in children and young adults, particularly males. Unlike other lymphomas, Burkitt's lymphoma has a specific geographic distribution: It is most common in central Africa and rare in the United States. The Epstein-Barr virus causes it, but it does not appear to be contagious. It is more common in people who have AIDS.

Burkitt's lymphoma grows and spreads quickly, often to the bone marrow, blood, and central nervous system. When it spreads, weakness and fatigue often develop. Large numbers of lymphoma cells may accumulate in the lymph nodes and organs of the abdomen, causing swelling. Lymphoma cells may invade the small intestine, resulting in blockage or bleeding. The neck and jaw may swell, sometimes painfully. To make the diagnosis, a doctor performs a biopsy of the abnormal tissue and orders procedures to stage the disease.

Without treatment, Burkitt's lymphoma is fatal. Surgery may be needed to remove affected parts of the intestine, which otherwise may bleed, become blocked, or rupture. Intensive chemotherapy can cure 70 to 80% of people if the disease has not spread widely. If the lymphoma has spread to the bone marrow, blood, or central nervous system at the time of diagnosis, the prognosis is much worse.

Symptoms

The first symptom is often painless enlargement of lymph nodes in the neck, under the arms, or in the groin. Enlarged lymph nodes within the chest may press against airways, causing cough and difficulty in breathing. Deep lymph nodes within the abdomen may press against various organs, causing loss of appetite, constipation, abdominal pain, or progressive swelling of the legs.

Since some lymphomas can appear in the bloodstream and bone marrow, people can develop symptoms related to too few red blood cells, white blood cells, or platelets. Too few red blood cells can cause anemia; and the person may have fatigue, shortness of breath, and pale skin. Too few white blood cells can lead to infections. Too few platelets may lead to increased bruising or bleeding. Non-Hodgkin's lymphomas also commonly invade the bone marrow, digestive tract, skin, and occasionally the nervous system, causing a variety of symptoms. Some people have persistent fever without an evident cause, the so-called fever of unknown origin. This commonly reflects an advanced stage of disease.

In children, the first symptoms—anemia, rashes, and neurologic symptoms, such as weakness and abnormal sensation—are likely to be caused by infiltration of lymphoma cells into the bone marrow, blood, skin, intestine, brain, and spinal cord. Lymph nodes that become enlarged are usually deep ones, leading to accumulation of fluid around the lungs, which causes difficulty in breathing; pressure on the intestine, which causes loss of appetite or vomiting; and blocked lymph vessels, which causes fluid retention, most noticeably in the arms and legs.

Symptoms of Non-Hodgkin's Lymphoma

Symptoms	Cause
Difficulty in breathing, swelling of the face	Enlarged lymph nodes in the chest
Loss of appetite, severe constipation, abdominal pain or distention	Enlarged lymph nodes in the abdomen
Progressive swelling of the legs	Blocked lymph vessels in the groin or abdomen
Weight loss, diarrhea, malabsorption (interference with	Invasion of the small intestine

digestion and passage of nutrients into the blood)	
Fluid accumulation around the lungs (pleural effusion)	Blocked lymph vessels in the chest
Thickened, dark, itchy areas of skin	Infiltration of the skin
Weight loss, fever, night sweats	Spread of the disease throughout the body
Anemia (an insufficient number of red blood cells)	Bleeding into the digestive tract, destruction of red blood cells by an enlarged spleen or by abnormal antibodies, destruction of bone marrow because of invasion by the lymphoma, inability of the bone marrow to produce sufficient numbers of red blood cells because of drugs or radiation therapy
Susceptibility to severe bacterial infections	Invasion of the bone marrow and lymph nodes, causing decreased antibody production

Diagnosis and Classification

Doctors perform a biopsy of an enlarged lymph node to diagnose non-Hodgkin's lymphoma and to distinguish it from Hodgkin's disease and other problems that cause enlarged lymph nodes.

Although more than 20 different diseases can be called non-Hodgkin's lymphoma, doctors sometimes group them into three broad categories. Indolent lymphomas are characterized by a survival of many years even when a person does not undergo treatment. Aggressive lymphomas are characterized by survival limited to several months in someone who goes untreated. Highly aggressive lymphomas are characterized by survival of only weeks when a person does not undergo treatment. Although non-Hodgkin's lymphomas are usually diseases of middle-aged and older people, children and young adults may develop lymphomas, and these lymphomas are commonly more aggressive.

Staging

Many people with a non-Hodgkin's lymphoma have disease that has spread at the time of diagnosis. In only 10 to 30% of people, the disease is limited to one specific area. People with the disease undergo similar staging procedures as those with Hodgkin's disease (see Lymphomas: Staging). In addition, a bone marrow biopsy is almost always performed.

Treatment and Prognosis

Almost everyone benefits from treatment. For some people, complete cure is possible; for others, treatment extends life and relieves symptoms for many years. The likelihood of cure or long-term survival depends on the type of non-Hodgkin's lymphoma and the stage when treatment starts. It is somewhat of a paradox that indolent lymphomas usually respond readily to treatment by going into remission (in which the disease is under control), often followed by long-term survival, but the disease usually is not cured. In contrast, aggressive and highly aggressive non-Hodgkin's lymphomas, which usually require very intensive treatment to achieve remission, have a good chance of being cured.

Stage I and II Non-Hodgkin's Lymphomas People with indolent lymphomas who have very limited disease (stages I and II) are often treated with radiation limited to the site of the lymphoma and adjacent areas. With this approach, 20 to 30% of people may have long-term remission and are probably cured. People with aggressive or highly aggressive lymphomas at a very early stage need to be treated with

combinations of chemotherapy, often with the addition of localized radiation therapy. With this approach, 70 to 90% of people are cured.

Stage III and IV Non-Hodgkin's Lymphomas Almost all people with indolent lymphomas have stage III or IV disease. They do not always require treatment, but they are closely monitored for evidence of complications that could signal more rapid progression of the disease. There is no evidence that early treatment in people with indolent lymphomas at more advanced stages extends survival. If the disease begins to progress more rapidly, there are many treatment choices.

Treatment may include chemotherapy with a single drug or as a combination of several different drugs. No treatment is clearly superior, so the choice of treatment is influenced by the extent of disease and the symptoms a person is having. Treatment usually produces a remission, but the average length of remission ranges from 2 to 4 years. A decision about treatment after a relapse (in which lymphoma cells reappear) again depends on the extent of the disease and the symptoms. After an initial relapse, remissions tend to become shorter.

Many new treatments are now available for indolent lymphomas. These include monoclonal antibodies, which bind to lymphoma cells and kill them. These antibodies (immunoglobulins), such as rituximab SOME TRADE NAMES RITUXAN, are given intravenously. Sometimes, the monoclonal antibodies are modified so that they can carry radioactive particles or toxic chemicals directly to the cancer cells in different parts of the body. It remains uncertain whether these monoclonal antibodies can cure non-Hodgkin's lymphomas, or if they can achieve better results when combined with chemotherapy.

Another new approach to treating indolent lymphomas involves vaccinating the person with proteins taken from his own lymphoma. The person's immune system recognizes the proteins as "foreign" and then fights the lymphoma in much the same way that it fights an infection.

For people with aggressive or highly aggressive stage III or IV non-Hodgkin's lymphomas, combinations of chemotherapy drugs are given promptly. Many potentially effective combinations of chemotherapy drugs are available. Combinations of chemotherapy drugs are often given names created by using single letters from each of the drugs that are included. For example, one of the oldest and still most

commonly used combinations is known as CHOP

cyclophosphamide SOME TRADE NAMES CYTOXAN, [hydroxy]doxorubicin (SOME TRADE NAMES ADRIAMYCIN), vincristine (SOME TRADE NAMES ONCOVIN) [Oncovin], and prednisone (SOME TRADE NAMES DELTASONE AND METICORTEN).

About 50% of people with aggressive or highly aggressive non-Hodgkin's lymphomas at an advanced stage are cured with CHOP chemotherapy. Newer combinations of drugs have not produced much improvement in cure rates. However, chemotherapy, which often causes different types of blood cells to decrease in number, is sometimes better tolerated if special proteins (called growth factors) are given to stimulate growth and development of blood cells. Chemotherapy for some people with aggressive or highly aggressive lymphomas is now combined with monoclonal antibodies. For example, results from the combination of CHOP with rituximab (SOME TRADE NAMES RITUXAN) may be better than from CHOP alone, but studies are still ongoing.

Chemotherapy at usual doses is of very limited value when relapse occurs. Many people who have a relapse of an aggressive or highly aggressive lymphoma at an advanced stage receive high-dose chemotherapy combined with autologous stem cell transplantation, involving the person's own stem cells (see Transplantation: Stem Cell Transplantation). With this type of treatment, up to 40% of people may be cured. Some stem cell transplants for people with an aggressive or highly aggressive lymphoma use stem cells from a matched or unrelated donor (allogeneic transplant), but this type of transplantation has a greater risk of complications.

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Non-Hodgkin's lymphoma: review of conventional treatments.

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The non-Hodgkin's lymphomas are a diverse groups of lymphoid neoplasms that collectively rank fifth in cancer incidence and mortality. Conventional treatment for patients with newly-diagnosed non-Hodgkin's lymphoma (NHL) includes radiation or chemotherapy. In addition, those with asymptomatic low-grade disease may follow a "watch and wait" approach. Single agent oral alkylating therapy and CVP (cyclophosphamide, vincristine, and prednisone) have become a mainstay of treatment for low-grade NHL. High intensity chemotherapy consisting of the anthracycline, doxorubicin along with cyclophosphamide, vincristine and prednisone (CHOP) is offered as standard treatment for intermediate-grade NHL. Following relapse, salvage therapy rarely results in long-term survival in patients with low-grade NHL. Up to 50% of patients die within five years of first relapse. For patients with intermediate-grade NHL who relapse after or do not respond to first-line treatment, a range of combination regimens can be offered, composed of non-cross resistant drugs not typically used during first-line treatment. However, less than half of patients with intermediate-grade disease achieve prolonged disease-free survival. With today's' conventional treatments, cure is only a possibility for a minority of patients with intermediate-grade disease and a limited group of patients with indolent NHL who are diagnosed at early stages. Novel approaches to treatment are therefore needed. Monoclonal antibodies may fulfill this need, administered either as single agents or in conjunction with conventional cytotoxic approaches. The task now lies in determining how best to use this new modality, with the hope of bringing a cure to a greater number of patients.

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Monoclonal antibodies in lymphoid neoplasia: principles for optimal combined therapy.

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Rituximab and other monoclonal antibody therapies now in development have the potential to markedly impact the treatment of non-Hodgkin's lymphoma (NHL). These agents have significant single-agent activity, distinct mechanisms of action, and, in the case of rituximab and other unconjugated antibodies, favorable toxicity profiles that are nonoverlapping with the adverse effects associated with conventional chemotherapy. These properties may allow for the use of novel combination therapies with enhanced outcomes for patients. Systematic evaluation of rationally designed combinations through randomized, prospective trials is required to determine the clinical utility of these novel agents and combinations will live up to their potential.

Epratuzumab, a Humanized Monoclonal Antibody Targeting CD22: Characterization of *in Vitro* Properties[1]

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Abstract

Purpose: **Epratuzumab is a novel humanized antihuman CD22 IgG1 antibody that has recently shown promising clinical activity, both as a single agent and in combination with rituximab, in patients with non-Hodgkin's lymphomas (NHL). In an attempt to better understand the mode of action of epratuzumab, the antibody was tested *in vitro* in a variety of cell-based assays similar to those used to evaluate the biological activity of other therapeutic monoclonal antibodies, including rituximab. In this report, we present epratuzumab activities as they relate to binding, signaling, and internalization of the receptor CD22.**

Methods: **Chinese hamster ovary-expressed CD22 extracellular domain was used to measure epratuzumab affinity on Biacore. CD22 receptor density and internalization rate were measured indirectly using a monovalently labeled, noncompeting (with epratuzumab) anti-CD22 antibody on Burkitt lymphoma cell lines, primary B cells derived from fresh tonsils, and B cells separated from peripheral blood samples obtained from patients with chronic lymphocytic leukemia or healthy volunteers. Epratuzumab-induced CD22 phosphorylation was measured by immunoprecipitation/Western blot and compared with that induced by anti-IgM stimulation.**

Results: **Epratuzumab binds to CD22-extracellular domain, with an affinity of K_D = 0.7 nM. Binding of epratuzumab to B cell lines, or primary B cells from healthy individuals and patients with NHL, results in rapid internalization of the CD22/antibody complex. Internalization appears to be faster at early time points in cell lines than in primary B cells and NHL patient-derived B cells, but the maximum internalization reached is comparable for all B cell populations after several hours of treatment and appears to reach saturation at antibody concentrations of 1–5 μg/ml. Finally, epratuzumab binding results in modest but significant CD22 phosphorylation.**

Conclusions: **Epratuzumab represents an excellent anti-CD22 ligating agent, highly efficacious in inducing CD22 internalization, and can induce phosphorylation. Although we cannot unequivocally demonstrate here that epratuzumab-induced internalization and signaling of CD22 directly contribute to its therapeutic efficacy, these properties are the fundamental characteristics of the target CD22 and its interaction with epratuzumab. Similar results were observed when epratuzumab was tested *in vitro* on Burkitt B cell lines as well as on primary normal B cells and neoplastic B cells separated from fresh peripheral blood samples from patients with chronic lymphocytic leukemia.**

Introduction

CD22 is a 135-kDa transmembrane sialoglycoprotein and a member of the immunoglobulin superfamily. Its expression is restricted to lymphocytes of the B cell lineage and is highly developmentally regulated: CD22 is present in the cytoplasm of pro- and pre-B cells and becomes detectable on the cell surface only at mature stages of B cell differentiation. Cell surface expression is lost during terminal differentiation into plasma cell and after B cell activation (1–3). CD22 is also expressed by the vast majority of B cell NHLs[3] (4). The CD22 molecule has multiple ligands because it binds to α2–6-linked sialic acid residues present on glycoproteins expressed by activated T and B cells, monocytes, neutrophils, erythrocytes, and activated endothelial cells (5). Although its function is not yet well understood, CD22 appears to be involved in the regulation of B cell activation through BCR signaling, (demonstrating both positive and negative roles *in vitro*) as well as in cell adhesion (6). *In vivo*, the important biological functions of this receptor have been demonstrated by genetic disruption of CD22. CD22-deficient mice have a shorter life span, a reduced number of mature B cells in the bone marrow and in circulation, and a chronic exaggerated antibody response to antigen and develop elevated levels of autoantibodies, suggesting a key role for CD22 in B cell development, survival, and function (1, 7–9).

Because the expression of CD22 is lineage restricted and, in most cases, is not lost during neoplastic transformation, it represents an attractive target for anti-NHL immunotherapeutic antibodies. Preclinical work with anti-CD22-"blocking" monoclonal antibodies (*i.e.*, monoclonal antibodies that prevent CD22

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[3] The abbreviations used are: NHL, non-Hodgkins lymphoma; BCR, B cell receptor; ECD, extracellular domain; PE, phycoerythrin; CLL, chronic lymphocytic leukemia.

binding to its natural ligands) suggests that modulation of receptor activity has a selective cytotoxic effect on receptor-positive tumor cells (10). Interestingly, the same ligand blocking anti-CD22 monoclonal antibody can trigger primary B cell proliferation, suggesting that the consequences of engaging CD22 may differ depending on the B cell stage of differentiation. In animal models, anti-CD22 monoclonal antibodies can mediate antitumor effects (11, 12). Unlike the B cell antigen CD20, CD22 internalizes rapidly upon antibody binding, making it an ideal target for delivery of radioisotopes or toxins to malignant cells via monoclonal antibody conjugates (11, 13–15).

LL2 is a mouse anti-CD22 monoclonal antibody (originally named EPB/2) generated against the Raji Burkitt's lymphoma cell line (16). LL2 is highly selective for B cell tumors but lacks reactivity with Hodgkin's disease, other solid tumors, or non-lymphoid tissues (17). A humanized version of the LL2 antibody, epratuzumab, was developed to minimize the potential for immunogenicity and enhancing effector interactions during its development as a diagnostic and immunotherapeutic (18). As a single agent, epratuzumab has shown preliminary evidence of antitumor activity in patients with recurrent NHL, producing responses, including durable complete responses (19). Objective responses were seen in 9 of 51 indolent NHL patients (17.6%; 3 complete responses, 6 partial responses) with a median duration of response of 47+ (range, 11–99+) weeks and median time to progression of 103+ (35–107+) weeks by Kaplan-Meier estimate. Of 52 aggressive NHL patients, 5 achieved objective responses (10%; 3 complete responses, 2 partial responses), with median duration of response 38+ (13 to 38+) weeks and median time to progression 35+ (23 to 35+) weeks. All responses occurred in the follicular NHL and diffuse large B cell histologies (19). Anti-CD22 agents are likely to have mechanism(s) of action distinct from those of other cytotoxic agents as well as from immunotherapies targeting other B cell antigens (*e.g.,* the anti-CD20 monoclonal antibody rituximab and anti-CD52 antibody alemtuzumab). Thus, they are theoretically good candidates for combination with other drugs in the treatment of B cell malignancies. Epratuzumab is currently being evaluated in combination with rituximab, and early results from an ongoing study suggest that the combination of the two antibodies is well tolerated and may result in improved clinical activity *versus* the single agents alone (20).

This paper reports the results of *in vitro* studies to further characterize the mechanism of action of epratuzumab by exploring its interaction with CD22.

Materials and Methods

Cell Lines and Reagents. The CD22-expressing human Burkitt's lymphoma cell lines, Daudi, Ramos, Namalwa, and Raji, were obtained from the American Type Culture Collection (Manassas, VA). The cell lines were grown in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated FBS (HyClone, Logan, UT), 2 mM glutamine,100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). Cells were cultured at 37°C in a humidified 5% CO_2 incubator. $F(ab')_2$ fragments of goat antihuman IgG(Fc) were purchased from Rockland Immunochemicals, Gilbertsville, PA. Other reagents included: $F(ab')_2$ fragment of goat antihuman IgM, Fc_{5u} (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), sodium orthovanadate (Sigma-Aldrich, St. Louis, MO), complete protease inhibitor mixture tablets (Roche, Mannheim, Germany), rabbit polyclonal anti-CD22 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), horseradish peroxidase-conjugated antiphosphotyrosine Ab (clone 4G10; Upstate Biochemicals, Inc., Lake Placid, NY), FITC, and PE-conjugated antihuman CD22 [clone SHCL-1 (Leu14)] antibody (BD Biosciences, San Diego, CA; PE:antibody ratio, 1:1), FITC-conjugated antihuman CD19 (BD Biosciences), and QuantiBRITE PE-conjugated beads (BD Biosciences).

Cloning of Soluble Human CD22 ECD. The extracellular domain of human β CD22 (amino acids 1–687) was amplified from a human spleen Quick Clone cDNA library (Clontech, Palo Alto, CA) using standard PCR procedure. The PCR primers contained the consensus Kozak sequences (CCACC) and the specific restriction enzyme sites for subsequent cloning linked to the 5′ and 3′ end of the CD22ECD sequences. The expression vector used, pDSRα, was modified from the pSRα vector (21), with a dihydrofolate reductase cassette inserted downstream to the expression cassette driven by the SV40 early promoter.

Expression and Purification of CD22 ECD in Mammalian Cells. Chinese hamster ovary cells (CHOd-) defective in dihydrofolate reductase, originally obtained from L. Chasin (Columbia University, New York, NY) were grown in complete medium (high-glucose DMEM supplemented with 5% FBS, 1% nonessential amino acids, 1% hypoxanthine-thymidine, and 1% glutamine-penicillin-streptomycin).

CHOd− cells were transfected with 10 μg/dish of linearized DNA [pDSRα CD22 ECD (containing amino acids 1–687 of human CD22)] using the calcium phosphate method (Invitrogen). CD22 ECD expression was analyzed by Western blot of serum-free conditioned medium harvested from confluent 24-well cultures. Gels were run under reducing conditions, and blots were probed with a rabbit polyclonal anti-CD22 antibody (Santa Cruz Biotechnology, Inc.). Clones showing the highest expression were expanded and seeded in roller bottles for the production of CD22 ECD.

CD22 ECD was purified from CHO cell-conditioned medium. The concentrated medium was buffer exchanged to 30 mM Tris-HCl, pH 8.5, and applied to a Q-Sepharose column equilibrated in the same buffer. The column was eluted with a linear gradient from 0 to 1 M NaCl in 30 mM Tris-HCl, pH 8.5. Fractions containing CD22 ECD were pooled and applied to a hydroxyapatite column equilibrated in 150 mM NaCl-30 mM Tris-HCl, pH 8.5. Bound protein was eluted with a linear gradient from 0 to 100 mM sodium phosphate, pH 7.0, in 150 mM NaCl. The fractions containing CD22 ECD were pooled and dialyzed into PBS. The final protein concentration was determined by UV absorbance at 280 nm, using an extinction coefficient of 144,270 M^{-1} cm^{-1}. The sample was determined to be ≥98% pure by SDS-PAGE.

Biacore Measurement of Epratuzumab Binding Affinity. Both kinetic and equilibrium analyses of epratuzumab were performed on a BIAcore 3000 (Biacore, Inc., Piscataway, NJ) with PBS and 0.005% P20 surfactant (BIAcore, Inc.) as running buffer.

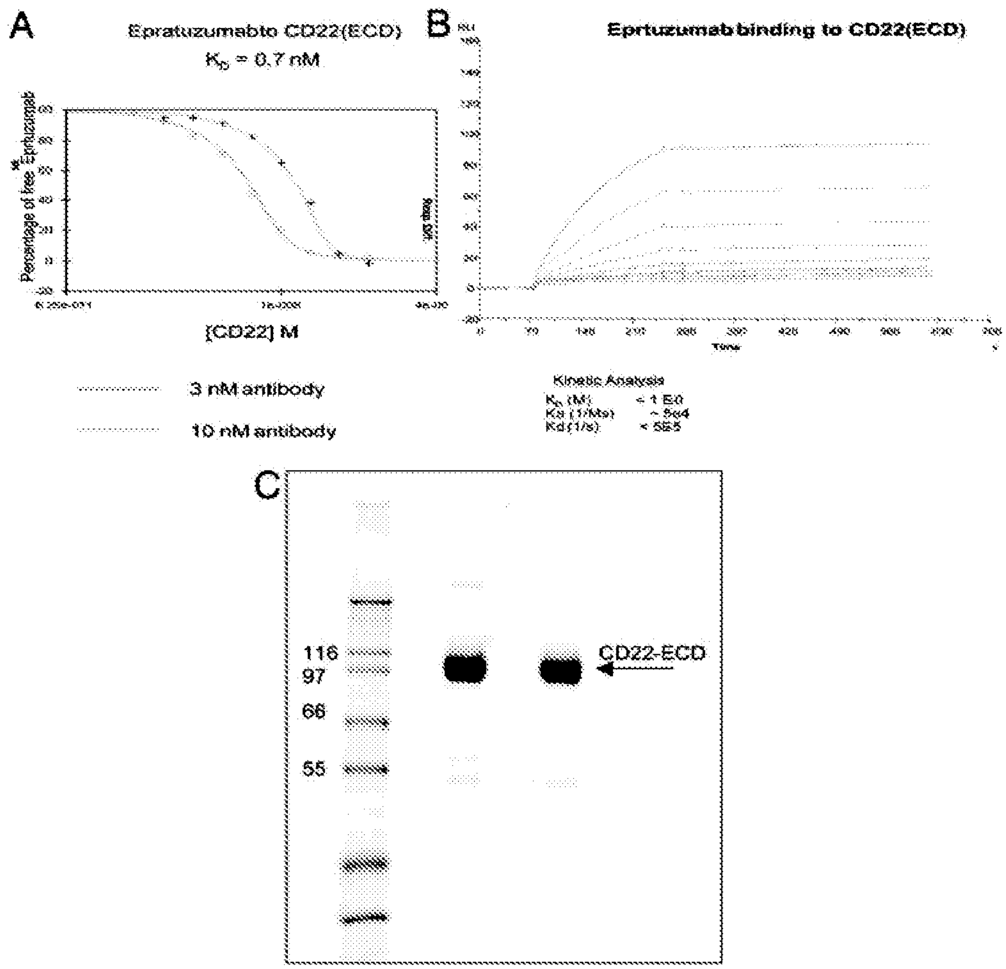

Fig. 1 Epratuzumab measured K_D on Biacore. Affinity of epratuzumab for CD22 ECD was determined by measuring binding of free epratuzumab to a Biacore column after competition with soluble CD22 ECD (*A*). B, kinetics of direct epratuzumab binding (0.8–200 nM) over a CD22 ECD surface, showing a very slow off rate. Cloned, Chinese hamster ovary-expressed, and purified CD22 ECD (as shown in panel *C*) on the blotted membrane with rabbit anti-CD22 was used for molecular characterization of epratuzumab binding.

To study the kinetics of epratuzumab/CD22 binding, CD22 ECD was immobilized to a research grade CM5 sensor chip (Biacore, Inc.) via primary amine groups using the Amine Coupling Kit (Biacore, Inc.) according to the manufacturer's suggested protocol. Resonance level was ~400 Ru. The analysis was effected by injection of increasing concentrations (0.78–200 nM) of epratuzumab over the CD22 surface at a flow rate of 50 μl/min for 3 min, and the dissociation reaction allowed for 15 min. Antibody binding kinetic parameters including k_a (association rate constant), k_d (dissociation rate constant), and K_D (dissociation equilibrium constant) were estimated using the BIA evaluation 3.1 computer program (BIAcore, Inc.).

To analyze the equilibrium constants of epratuzumab-CD22 binding, CD22 ECD was immobilized to a CM5 chip (Resonance level = 2300 Ru) according to the manufacturer's suggested protocol. Epratuzumab at two different concentrations (3 nM and 10 nM) was incubated with increasing concentrations (0.04 nM to 80 nM) of CD22 in sample buffer (PBS + 0.005% P-20 + 0.1 mg/ml BSA) for >2 h to allow samples to reach equilibrium. Samples were then injected over the CD22 surface at 10 μl/min for 30 min. In this system, the binding signal obtained is proportional to the free antibody in solution at equilibrium. The dissociation equilibrium constant (K_D) was obtained from nonlinear regression analysis of the competition curves using a dual-curve one-site homogeneous binding model (KinExA software; Sapidyne Instruments, Inc., Boise, ID).

CD22 Immunoprecipitation and Western Blot Analysis. A total of 10^7 Daudi or Ramos cells (in 15 ml of RPMI 1640 plus 10% FBS) were treated with epratuzumab (5 μg/ml); epratuzumab (5 μg/ml) plus a cross-linking antibody (antihuman IgG(Fc) or $F(ab')_2$ fragments at 5 μg/ml[or antihuman IgM $F(ab')_2$ fragments at 5 μg/ml at 37°C for 15 min. Cells without antibody treatment were used as a negative control. After two washings with cold PBS, cells were centrifuged at 200 × g at 4°C for 8 min and resuspended in 400 μl of lysis buffer (1% Triton X-100, 0.1% SDS in 1× PBS with 2 mM sodium orthovanadate and protease inhibitor mixture). Cells were sonicated, kept on ice for 30 min, and centrifuged at 16,000 × g at 4°C for 10 min. Epratuzumab (3 μg) and 20 μl of protein G^+/protein A-agarose beads (Oncogen Research Products, Boston, MA) were added to each supernatant and agitated overnight. Beads were then washed three times with lysis buffer. The immunoprecipitated proteins were size fractionated on SDS-PAGE and transferred to nitrocellulose. Membranes were analyzed by immunoblotting with horseradish peroxidase-conjugated antiphosphotyrosine antibody, treated with

stripping buffer (Pierce, Rockford, IL), and reprobed with a rabbit polyclonal anti-CD22.

Primary B Cells from Fresh Tonsils and CLL Samples. Tonsils were collected according to standard surgical procedures, including obtaining patient informed consent, and were shipped overnight in RPMI containing penicillin and streptomycin. Cells were gently mechanically dissociated in cold Hanks' balanced salt solution (Invitrogen), and mononuclear cells were purified using Ficoll-Hypaque (Amersham Biosciences) according to specification. Mononuclear cells were resuspended in Hanks' medium and washed with RPMI containing 10% FBS. Whenever the B cell:total cell ratio fell below 60%, further purification was performed using a T cell depletion kit from Dynal Biotech (Oslo, Norway).

Fresh CLL blood samples were collected in heparinized tubes from patients who had provided informed consent and were shipped overnight on ice. Ficoll-Hypaque purification of mononuclear cells was then performed as previously described. Cells were then immunostained with anti-CD19, anti-CD20, or anti-CD22 as described for FACS analysis and receptor quantification.

Confocal Microscopy Study of Direct Epratuzumab Internalization. FITC-labeled epratuzumab (2 μg/ml) was used to label Daudi cells (10^6 cells/ml) in PBS plus 0.5% BSA, on ice for 30 min. Cells were subsequently washed twice in PBS plus 0.5% BSA, plated in four-well chamber coverslips (Nalge Nunc International), and allowed to incubate for an additional 60 min at either 4°C or 37°C. Confocal images were recorded using an ACAS Ultima confocal microscope (Meridian Instruments, Inc., Okemos, MI) and represent 1-μm sections through the center of a focal plane using a 100× oil immersion objective.

CD22, CD20, and CD19 Receptor Density Quantification. Burkitt's lymphoma cell lines, enriched mononuclear cells from freshly dissociated tonsils, CLL, or normal healthy volunteer peripheral blood samples were used. One million cells per sample were blocked for nonspecific staining with 2% FBS + 1% human serum in PBS (0.05% sodium azide) for 20 min on ice. One microgram of anti-CD19-PE (clone SJ25C1), anti-CD20-PE (clone L27), or anti-CD22-PE [clone S-HCL-1 (Leu-14)] monoclonal antibody with the appropriate isotype controls was added for 30 min at 4°C in the dark. All antibodies were from BD Biosciences. Samples were washed with 2 ml of HBSS and centrifuged at 500 × *g* for 8 min at 10°C. The supernatants were discarded, and the cells were resuspended in 0.5 ml of FACS Lysing Solution (BD Biosciences) and immediately analyzed by flow cytometry (BD FACSCalibur). The CD19, CD20, and CD22 receptor numbers were converted from the standard fluorescence curve set by the QuantiBRITE PE fluorescence quantitation kit (BD Biosciences).

Results

Epratuzumab Binds to CD22 ECD on Biacore with a Measured Affinity of K_D = 0.7 nM. The binding activity of epratuzumab to CD22 ECD was measured on Biacore. In this system, in which CD22 ECD is immobilized, epratuzumab shows a relatively slow off rate ($K_{off} < 10^{-5}$ 1/s; Fig. 1*B*), leading to a K_D of 0.7 nM (Fig. 1*A*) as determined by equilibrium analysis. Although the on rate (k_a) for epratuzumab is relatively slow, the resulting K_D is highly comparable with the reported 8 nM for rituximab (rituximab package insert).

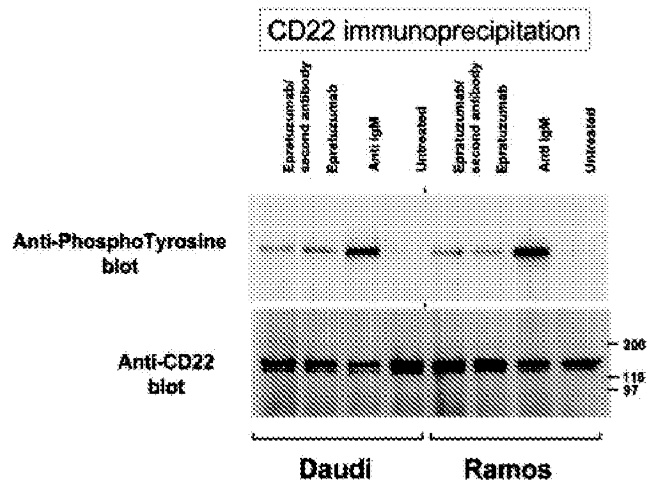

Fig 2 Epratuzumab induces phosphorylation of CD22. Daudi or Ramos cell lines were treated for 15 min at 37°C with a saturating dose of epratuzumab, epratuzumab and a cross-linker, or a B cell receptor activator, anti-IgM. Cells were lysed and CD22 was immunoprecipitated to examine the signaling potential of epratuzumab, using phosphotyrosine-specific antibody (*top*). To ensure equal CD22 loading, immunoblots were stripped and reprobed with a polyclonal rabbit anti-CD22 (*bottom*) (see "Materials and Methods").

Epratuzumab Binding Results in CD22 Phosphorylation. CD22 ligation has been reported by others to induce phosphorylation on its intracytoplasmic tail (10). Tyrosine phosphorylation of the CD22 cytoplasmic tail is also induced by BCR cross-linking. We therefore attempted (*a*) to detect whether epratuzumab could induce CD22 signaling and (*b*) to compare it with the CD22 tyrosine phosphorylation induced by B cell activation, using anti-IgM stimulation (Fig. 2).

Daudi and Ramos Burkitt's lymphoma cell lines were treated for 15 min with saturating concentrations of epratuzumab. Immunoprecipitation-Western blot showed a significant increase in CD22 phosphorylation in both cell lines. Cross-linking epratuzumab using a secondary antibody did not result in higher level of phosphorylation. As expected, stimulation of the BCR using an activating anti-IgM antibody resulted in the highest CD22 phosphorylation level. Epratuzumab binding to tonsil-derived primary B cells also resulted in CD22 phosphorylation above resting level. However, the CD22 phosphorylation level in unstimulated tonsil cells was higher than in the unstimulated cell lines, decreasing the signal:noise ratio (data not shown).

Epratuzumab Internalization Visualized by Confocal Microscopy. In an effort to visually document the rapid, direct internalization of epratuzumab after binding to CD22, epratuzumab was directly labeled with FITC, and its binding to Daudi Burkitt lymphoma cells was monitored at 37°C during 60 min. As a control, Daudi cells were exposed to the same epratuzumab concentration but kept on ice to prevent internalization (Fig. 3, *left*). FITC-epratuzumab/CD22 internalization is evident from capping phenomena and punctate staining of endocytic vesicles (Fig. 3, *arrows*) visible in cells incubated at 37°C only.

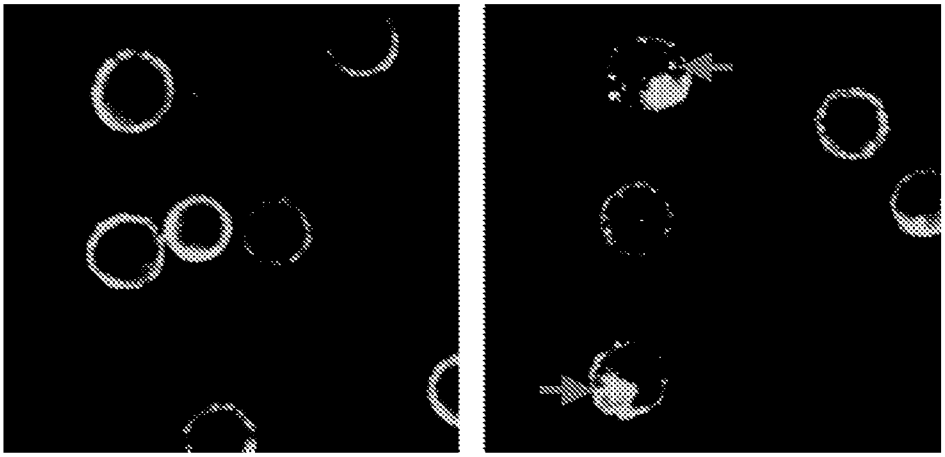

Fig. 3 FITC-epratuzumab direct internalization. FITC-labeled epratuzumab was used to label Daudi tumor cells on ice for 30 min. Cells were subsequently washed and allowed to incubate for an additional 60 min at either 4°C (*left*) or 37°C (*right*). CD22 internalization is evident from capping and punctate staining of endocytic vesicles (*arrows*) visible in cells incubated at 37°C.

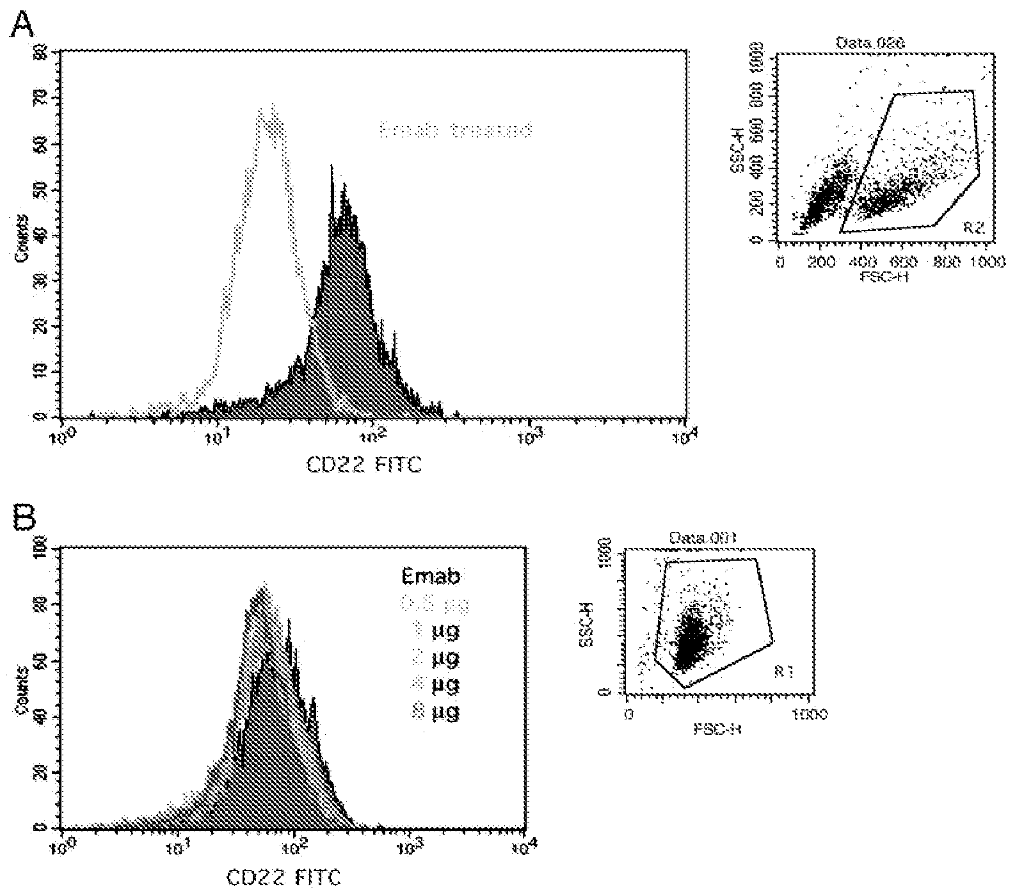

Fig. 4 CD22 internalization as measured by flow cytometry using a noncompeting anti-CD22 antibody. Daudi cells were incubated in the presence of 5 µg/ml epratuzumab (*Emab*) for 20 h at 37°C and analyzed by flow cytometry for CD22 internalization (*A*) as observed by an apparent lowering of cell surface CD22, measured with the FITC-S-HCL1 anti-CD22 monoclonal antibody. B, evidence that adding increasing epratuzumab concentrations together with FITC-labeled S-HCL1 did not result in lowering the signal observed with FITCH-SCI1 alone (*solid blue trace*). This confirms that these two anti-CD22 monoclonal antibodies recognize distinct and noncompeting epitopes on CD22 (FACS using the Daudi cell line). *FSC-H*, forward scatter; *SSC-H*, side scatter.

Internalization of Epratuzumab Results in Quantifiable CD22 Cell Surface Decrease. One particularly intriguing property of CD22 is its capacity to rapidly translocate from the cytoplasm to the cell surface on B cell stimulation on cell lines, (22) and to rapidly internalize upon antibody ligation (23). The result of such internalization is an apparent reduction in the number of cell surface CD22 binding sites (Fig. 4*A*), measurable using a noncompeting anti-CD22 (anti-CD22-PE [clone S-HCL-1 (Leu-14)] monoclonal antibody (24).

We confirmed that the anti-CD22 [clone S-HCL-1 (Leu-14)] monoclonal antibody did not compete with epratuzumab for CD22 binding and therefore could be used as a tool to independently measure cell surface CD22. As shown in Fig. 4*B*, increasing concentration of epratuzumab added together with S-HCL1-FITC labeled antibody did not result in a displacement of the signal observed with S-HCL1-FITC antibody alone.

To quantify cell surface CD22, the S-HCL1 antibody monovalently labeled with PE (conjugated by BD Biosciences)

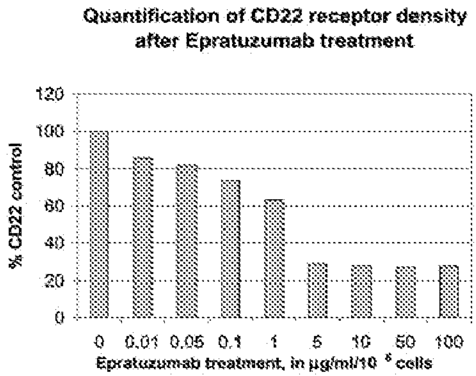

Fig. 5 Quantification of CD22 receptor density after epratuzumab treatment. Daudi lymphoma cells (10^6 cells/ml) were incubated with increasing concentrations of epratuzumab in RPMI supplemented with 10% FBS for 1 h at 37°C. Cells were then washed with cold PBS and immediately analyzed by FACS for CD22 receptor quantification as described. The resulting CD22 density as measured with QuantiBRITE beads and H-SCL1 PE labeled is shown as a percentage of total CD22 on untreated cells (100%). At 5 µg/ml, cells have reached the maximum level of internalization, which is 70% in this experiment.

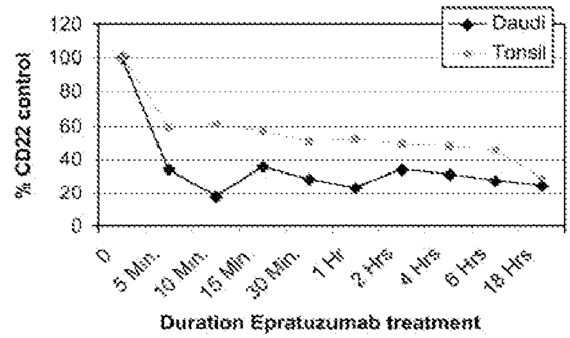

Fig. 6 Epratuzuma-induced CD22 internalization kinetics. Daudi cells and tonsil-derived mononuclear cells were incubated with increasing incubation time in the presence of 25 µg/ml epratuzumab/ At the end of each time point, cells were immunostained with anti-CD22 H-SCL1-PE only (Daudi) or anti-CD19-FITC and anti-CD22-PE (tonsils) for receptor quantification, as described. At early time points, the Daudi cell line is more efficacious in receptor internalization. After prolonged treatment, however (>18 h), no internalization efficiency difference between cell lines and primary cells could be observed.

was then used after cells were incubated with epratuzumab. This allowed indirect measurement of the CD22 receptor internalization rate and extent after a variety of treatments, without direct modification of epratuzumab.

Incubation of Daudi cells for 1 hour with epratuzumab resulted in a dose dependent increase in CD22 internalization (Fig. 5). The point of saturation was reached between 1 to 5 µg/ml/10^6 cells (31.5 nM). At this time point, 30% of the original CD22 density (or 70% internalization) was observed. Similar results were obtained with other cell lines, such as Ramos, Raji, and Namalwa (data not shown), suggesting that the lack of total internalization may reflect more the endogenous turnover of the receptor (25) rather than expression of another CD22 isoform (*i.e.,* the α isoform of CD22, lacking domains 3 and 4 and therefore presumably not bound by epratuzumab).

Consistent with a dynamic equilibrium between rapid epratuzumab-induced CD22 internalization and trafficking to the cell surface of new receptor, prolonged incubation time with epratuzumab resulted in higher degree of internalization, reaching close to 80% after an overnight incubation, in both cell lines (Daudi) and primary tonsil-derived B cells (Fig. 6). At early time points, the Daudi cell line internalizes CD22 very rapidly, consistent with results reported previously for the Raji cell line (23). Internalization of the epratuzumab/CD22 complex on tonsil-derived primary B cells appears much slower. However, the extent and trend for increasing internalization over time did not differ significantly between the two cell populations after a 30-min incubation. To determine whether these observations could be extended to "fresh" lymphoma cells, mononuclear cells isolated by Ficoll-Hypaque from peripheral blood samples from patients with CLL were incubated with saturating concentrations of epratuzumab for 2 h, and CD22 expression was quantified at the end of the incubation time using the QuantiBRITE system (Table 1). Among the CLL and NHL samples tested, CD22 receptor density tends to be lower than in established lymphoma cell lines. However, even in these samples, evidence of CD22 internalization induced by epratuzumab was obtained after a 2-h incubation. For comparison, we tested in the same experiment the B lymphoma cell line Namalwa that expresses low levels of cell surface CD22. Namalwa cells, like Daudi cells, show a very fast and almost maximum level of internalization within minutes (data not shown), indicating that epratuzumab-mediated receptor internalization can occur very efficiently even with low receptor density, but the kinetics may differ significantly between cell lines and primary samples.

CD22 Reexpression after Epratuzumab Treatment. Because in the clinical setting patients will be exposed to epratuzumab for days ($t_{1/2}$ = 23 days), the effect of longer epratuzumab exposure was also explored on Daudi and Ramos cell lines by growing them in the presence of 10 µg/ml epratuzumab for up to 13 days. At each passage/feeding time, cells were counted, and viability was evaluated with trypan blue. No significant difference in cell density and viability could be detected between epratuzumab-treated or control cells (data not shown), suggesting that epratuzumab does not have a direct cytotoxic or cytostatic effect *in vitro*. At day 4 or 7 during the 13-day period, some cells were washed and transferred to non-epratuzumab-containing medium and cultured for an additional 9 or 6 days, respectively. On the last day, all groups of cells were stained with FITC-H-SCL1 anti-CD22 monoclonal antibody and analyzed by flow cytometry (Fig. 7). As shown in Fig. 7, prolonged exposure of cells to epratuzumab for up to 13 days did not result in total abrogation of CD22 cell surface staining. Surprisingly, both Daudi (Fig. 7, *top*) and Ramos (Fig. 7, *bottom*) cell lines did not show full recovery of CD22 expression even after a prolonged (6 and 9 days) period of recovery from epratuzumab treatment when compared with cells grown in control conditions without epratuzumab.

Discussion

CD22 is an appealing target for the development of novel treatments for B cell malignancies because of its expression, which is B cell restricted and developmentally regulated, and its

Table 1 CD22, CD20, and CD19 receptor density, and epratuzumab-induced CD22 internalization on NHL and tonsil primary B cells-derived compared to two Burkitt lymphoma cell lines

Patient	Samples	Receptor density			% internalization
		CD22	CD19	CD20	
1	CLL	1,037	ND[b]	ND	50
2	CLL	2,077	4,770	4,230	40
3	NHL	1,797	3,859	6,758	ND
4	Mantle cell lymphomas	16,907	ND	ND	65
5	CLL	6,107	ND	ND	48
6	Normal tonsils	11,326	3,482	12,485	40–50
7	Normal tonsils	5,570	3,438	40,930	40–50
8	Normal tonsils	6,574	8,201	15,584	40–50
9	Healthy donor	20,047	ND	29,312	ND
	Daudi	22,548	21,787	25,604	60
	Nawalma	5,187	6,309	3,584	50

[a] Primary B cells were enriched from freshly dissociated tonsils (sample 6–8) or blood collected from patients with B-cell malignancies (sample 1–5) or healthy volunteer (sample 9). Cells were immediately analyzed for receptor density quantification as described, or treated with saturating dose of epratuzumab, for 2 hours at 37°C.

[b] ND, not done.

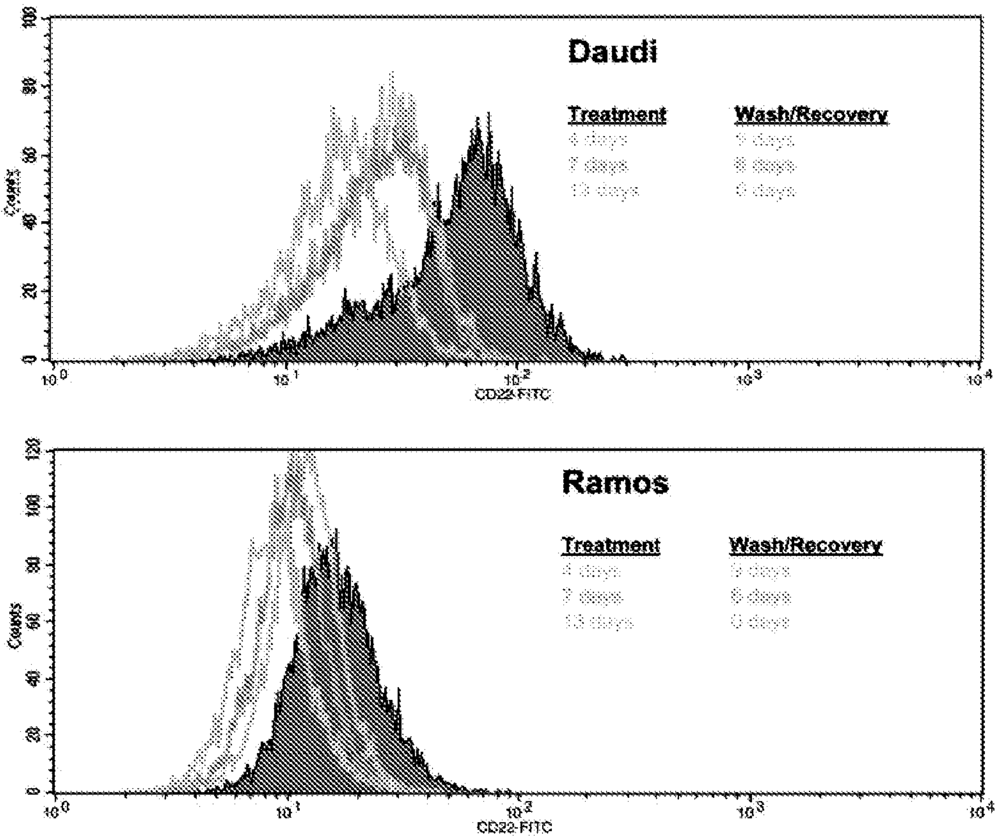

Fig. 7 Partial recovery of surface CD22 after epratuzumab treatment. Daudi or Ramos cell lines were grown in RPMI-10% FBS with added 20 μg/ml epratuzumab (or no added antibody for negative control) for up to 13 days. After 4 or 7 days treatment, cells were washed and allowed to recover in non-antibody-containing growth medium for 6–9 days. In all, the experiment lasted for 13 days, during which cell density was kept at 10^6 cells/ml, and viability was >95%. At the end of 13 days, all groups were analyzed by flow cytometry for CD22 expression using H-SCL1-FITC labeled (BD Biosciences), and the profiles were overlaid with that of untreated control cells (*solid blue trace*).

physiological functions as an adhesion molecule and a regulator of B cell receptor activation. One of the main functions of CD22 is to regulate B cell responses through recruitment of key signaling molecules to the antigen/receptor complex. Part of the BCR activation pathway includes tyrosine phosphorylation of the CD22 intracellular tail. However, CD22 ligand-mediated signaling can also be achieved by engaging the CD22 ligand binding site (mapped to CD22, domains 1 and 2) (5).

Epratuzumab is a humanized monoclonal antibody directed against the CD22 molecule; the parental murine antibody (LL2) was originally developed by Goldenberg *et al.* by "classic" immunization of mice with Raji cells as a source of B cell

antigens. This report confirms unequivocally that epratuzumab binds to CD22 ECD. The epratuzumab K_D value measured in this study on Biacore is consistent with previously published data on epratuzumab binding affinity to Raji cells using radiolabeled LL2 competed out with cold LL2 (estimated K_D from these experiments would be in the low nM range) (26). This compares favorably with other monoclonal antibodies used in the treatment of B cell lymphomas; for instance, rituximab affinity is reported to be 8 nM (rituximab package insert).

Epratuzumab binding to CD22 does not block the ligand-binding site on CD22 (17). In fact, as opposed to the ligand blocking anti-CD22 monoclonal antibodies (10), epratuzumab did not show evidence of inducing apoptosis or growth arrest on lymphoma cell growth *in vitro* (data not shown). Because CD22 interacts with a variety of ligands (including CD45) and cellular partners (including T cells) (5, 27), the effect of modulating this receptor should be significant *in vivo* but might be difficult to demonstrate in an isolated context *in vitro*. For example, others have shown that blocking CD22 translocation by using beads coated with anti-CD22 antibodies can alter the B cell threshold of activation thorough BCR (28).

In addition to its signaling regulation of B cell activation, CD22 was shown by several groups to internalize rapidly on antibody ligation (29). In the majority of cases internalization was demonstrated using radiolabeled antibody. In this report, we confirm the results previously published by using FITC-labeled epratuzumab as well as by measuring "naked" epratuzumab binding/internalization indirectly. In particular, in our system using confocal microscopy the internalization of the epratuzumab/CD22 complex could be visualized as intracytoplasmic vesicles presumably of endosome-lysosome origin. The internalization was dose dependent and long lasting, without reaching reversibility at clinically meaningful concentrations. Moreover, epratuzumab prolonged incubation results, at a given dose, in a progressive decrease in CD22 surface level, presumably shifting the equilibrium between internalization and receptor turnover. However, total disappearance of CD22 from the cell surface was never observed. These results could be explained by the known coexpression of a shorter spliced variant of CD22, anti-CD22. This spliced mRNA minor variant found on human B cells is missing exons 5–8, resulting in CD22 ECD that lack domains 3 and 4 (30). This isoform therefore would not bear the epitope to which epratuzumab binds. However, using cell lines known to express various ratios of the two isoforms, similar apparent incomplete depletion of cell surface CD22 (data not shown) after prolonged epratuzumab incubation was observed, thus making the above hypothesis unlikely. Because in the clinical setting patients will be exposed to epratuzumab for a long period of time, we are now measuring the effect of epratuzumab treatment on CD22 mRNA expression as well as on time for complete recovery of cell surface CD22.[4] Reexpression of CD22 after prolonged epratuzumab treatment appears to follow slow kinetics; this was determined on two B cell lines grown for a different number of days in antibody-free medium after being exposed to epratuzumab for various periods of time. To our surprise, even after cells were washed and grown for 4–9 more days in what should be essentially epratuzumab-free medium, the CD22 level did not fully recover compared with untreated cells. It is possible that the actual epratuzumab concentration in fresh medium is modified by the epratuzumab pool bound to cytoplasmic CD22 being released during cell division or that CD22 expression at the RNA level is down-regulated by the antibody treatment. This is an important point to address in future experiments, because it may have implications in epratuzumab dosing/scheduling in the clinic.

In conclusion, the CD22 molecule serves at least two important roles, *i.e.*, regulation of BCR activation (mostly negative) and involvement in mature B cell homing. Both are key functions that epratuzumab can modulate through induction of CD22 phosphorylation and CD22 internalization, respectively. Although difficult to demonstrate *in vitro*, these effects could have very different implications depending on the stage of maturation of B cells, and their compartment localization. For example, recent reports suggest adhesion molecules to be implicated in homing, dissemination, and survival of NHL cells (31, 32).

Overall the results presented here, although not directly answering the question of the *in vivo* mechanism of action of epratuzumab, do help to clearly differentiate this antibody from other monoclonal antibodies currently used in the treatment of NHL. This differentiation, at least theoretically, forms the basis for the clinical evaluation of combination therapies.

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Epratuzumab, a CD22-targeting recombinant humanized antibody with a different mode of action from rituximab

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Abstract

Epratuzumab is a humanized anti-CD22 monoclonal antibody currently in clinical trials for treatment of non-Hodgkin lymphoma (NHL) and certain autoimmune diseases. Here we report the results of investigations of epratuzumab's mode of action in comparison to and in combination with the anti-CD20 mAb, rituximab. *In vitro* cell growth inhibition, induction of apoptosis, and the ability of the mAbs to mediate complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) were evaluated. We also investigated the potential activity of epratuzumab in the regulation of B-cell antigen receptor (BCR) activation. Epratuzumab and rituximab displayed very distinct modes of action; epratuzumab acts as an immunomodulatory agent, while rituximab is an acutely cytotoxic therapeutic antibody. Epratuzumab has distinct effects on cell growth from rituximab. For example, rituximab + anti-human IgG Fcγ yielded marked inhibition of proliferation in human NHL cell lines, while epratuzumab had little or no effect in this assay. However, when cells were immobilized and stimulated with anti-IgM, epratuzumab, but not rituximab, caused a significant antiproliferative effect. Unlike rituximab, no CDC could be detected, and ADCC was modest but significant with epratuzumab. Importantly, combining rituximab and epratuzumab did not decrease rituximab's ability to induce apoptosis, CDC, and ADCC. In fact, the combination is more effective than rituximab alone in inhibiting proliferation of Daudi Burkitt lymphoma cells in the presence of second antibody, and at least equally effective to rituximab in the absence of crosslinking. These observations suggest that it may be possible to enhance clinical efficacy by combination therapy comprised of anti-CD20 and anti-CD22 mAbs.
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Keywords: CD22; CD20; Epratuzumab; Rituximab; Cytotoxicity

1. Introduction

The development of therapeutic monoclonal antibodies (mAbs) has become an active and fruitful area of research. MAb therapy has been found particularly effective in non-Hodgkin lymphoma (NHL), presumably because B cells express unique cell surface targets. Rituximab, a murine-human chimeric anti-CD20 mAb, was the first such agent to obtain FDA approval and widespread clinical use (McLaughlin et al., 1998; Reff et al., 1994). Based on *in vitro* studies, the mechanisms of action of rituximab include complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC), as well as direct induction of apoptosis (Cartron et al., 2004). Recent investigations highlight the potential role of rituximab in chemosensitization, possibly through an impairment of intracellular signaling (Mounier et al., 2003).

Epratuzumab, a humanized IgG1 mAb targeting the cell surface antigen, CD22, has also demonstrated therapeutic efficacy against NHL in clinical trials (Leonard et al., 2003) as well as certain autoimmune diseases (Kaufmann et al., 2004; Steinfeld et al., 2005). A striking property of epratuzumab is that binding to B-lymphocytes results in rapid internalization (within minutes) of the cell surface CD22 (Carnahan et al., 2003), making epratuzumab an excellent agent for tumor-imaging (Baum et al., 1994), and for developing immunoconjugates with cyto-

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toxic drugs (Qu et al., 1998), toxins (Kreitman et al., 1993), or radionuclides (Linden et al., 2005). Epratuzumab has also been reported to induce CD22 phosphorylation, indicating a role in signal transduction (Carnahan et al., 2003). Similar to CD20, CD22 exhibits a highly restricted expression pattern, present only on B lymphocytes (Dorken et al., 1989). CD22 belongs to a class of membrane receptors that are known to modulate B-cell antigen receptor (BCR) activation. It is believed that BCR activation through antigen engagement results in a signaling cascade mediated through phosphorylation of the BCR, involving the MAP kinase pathway, as well as phosphorylation of its positive and negative regulators, CD19 and CD22, respectively. Phosphorylation of CD22 results in SHP-1 phosphatase translocation in proximity to the activated BCR, providing negative feedback (Tedder et al., 2005). The role of CD22 as a negative regulator of signaling is also suggested by the hyperactivated or chronic activation state of the B cells in CD22 genetically deleted murine models (Nitschke et al., 1997; O'Keefe et al., 1996; Otipoby et al., 1996; Sato et al., 1996).

Based on the involvement of CD22 with the BCR, its apparent role as a negative modulator, and the less dramatic B lymphocyte depletion (compared to that of rituximab) observed clinically (Leonard et al., 2003), we investigated the effects of epratuzumab in cell-based assays. Here we report the results of the investigations of epratuzumab's mode of action in comparison to and in combination with rituximab. We found that the two antibodies show very distinct modes of action, with epratuzumab appearing more as an immunomodulatory agent, in addition to its cytotoxic role, and rituximab acting as an acutely cytotoxic therapeutic antibody. We observed that combining both antibodies would not be detrimental to the cytolytic effects of rituximab.

2. Materials and methods

2.1. Cell lines

The CD22-expressing human Burkitt lymphoma cell lines, Daudi, Ramos, and Raji, were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Cell lines RL and SU-DHL-6, which contain the chromosomal translocation *t*(14;18), were obtained from Dr. John Gribben (Dana-Farber Cancer Institute, Boston, MA) and Dr. Alan Epstein (University of Southern California, Los Angeles, CA), respectively. Cell lines SU-DHL-4, SU-DHL-10, and Karpas422 were provided by Dr. Myron Czuczman (Roswell Park Cancer Institute, Buffalo, NY). The cell lines were grown in RPMI-1640 medium (Invitrogen, Carlsbad, CA) or DMEM (Life Technologies, Gaithersburg, MD), supplemented with 10% heat inactivated fetal bovine serum (FBS) (HyClone), 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

2.2. Immunophenotyping

Indirect immunofluorescence assays were done using FITC-goat anti-human IgG, Fcγ fragment specific antibody (Tago, Inc., Burlingame, CA) essentially as described previously (Stein et al., 1989) and analyzed by flow cytometry using a FACSCaliber (Becton Dickinson, San Jose, CA).

2.3. Primary B cells from fresh human tonsils

Tonsils were collected according to standard surgical procedures, after obtaining informed consent, and were shipped overnight in RPMI-1640 medium containing penicillin and streptomycin. Cells were dissociated mechanically in cold Hanks' Balanced Salt solution (Invitrogen, Carlsbad, CA), and mononuclear cells purified using Ficoll-Hypaque™ (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's specifications. Mononuclear cells were resuspended in Hanks' medium, and washed using RPMI medium containing 10% FBS. B cells were further purified using a T-cell depletion kit (Dynal Biotech, Oslo, Norway), according to the manufacturer's directions.

2.4. In vitro cell proliferation assays

2.4.1. Effects of mAbs on ^{3}H-thymidine uptake

MAb effects on cell growth were determined by measuring ^{3}H-thymidine incorporation in the NHL cell lines with and without the presence of a crosslinking second antibody, essentially as described by Shan et al. (1998). Specificity of the effect was assessed by comparison to the isotype-matched control and the stimulatory effect of the goat anti-mouse versus goat anti-human second mAbs for humanized (or chimeric) mAbs. All tests were performed in triplicate.

2.4.2. BCR stimulation

Ninety-six well tissue culture plates (black well; Corning Inc., Corning, NY) were coated for 48 h with epratuzumab or rituximab in PBS, pH 7.4, at 4 °C at the indicated concentrations. Plates were then washed once with cold PBS, then once with RPMI, 10% FBS. The cell lines were plated at a density of 30,000 cells per well. Anti-IgM $Fc_{5\mu}$ (Jackson ImmunoResearch Lab, Inc., West Grove, PA) was used at doses ranging from 0.1 to 10 μg/ml for 48 h. Cell viability was measured by addition to each well of 15 μl Alamar Blue (Biosource, Camarillo, CA), and read on a fluorescence plate reader (Spectramax Gemini, Molecular Devices, Sunnyvale CA) with an excitation of 530 nm and an emission of 590 nm. Reading was normalized by subtracting signal in wells containing tissue culture medium but without plated cells (0%).

2.5. Cytotoxicity assays

^{51}Chromium-release assays were performed for the measurement of ADCC essentially as described (Cardarelli et al., 2002). Effector cells were prepared from peripheral blood collected under a protocol approval by an institutional review board and in accordance with an assurance filed with and approved by the Department of Health and Human Services. Informed consent was obtained from each subject. CDC was measured using Alamar Blue according to Gazzano-Santoro et al. (1997).

2.6. Apoptosis quantification

2.6.1. Annexin V–FITC

Cells (2×10^5/ml) were incubated at 37 °C with 2 μg/ml of test antibody with or without the crosslinker, protein G (2 μg/ml), or a secondary antibody, $F(ab')_2$ fragment of a goat anti-human IgG Fcγ-specific antibody (Rockland Immunochemicals, Gilbertsville, PA) for up to 48 h. Cells were then washed twice with cold PBS, and incubated with Annexin V-FITC and propidium iodide (PI), according to the manufacturer's protocol (BD Biosciences, San Jose, CA), and analyzed by flow cytometry on a Becton Dickinson FACSCalibur. Untreated cells were used as control to gate Annexin V and PI negative cells. The percent of apoptotic cells was calculated by including all Annexin V-FITC positive cells.

2.7. Analysis of hypodiploid DNA by PI staining

Hypodiploid DNA was assesses after PI staining as described previously (Stein et al., 2004). Samples were analyzed by flow cytometry using a FACSCaliber. Percent apoptotic cells was defined as the percent of cells with DNA staining before G1/G0 peak (hypodiploid).

3. Results

3.1. Antigen expression of cultured human lymphoma cell lines

Flow cytometry analysis was performed using indirect immunofluorescent staining to confirm that epratuzumab and rituximab bind to a panel of cultured human B-cell lymphomas. As shown in Table 1, these mAbs bind to the tested cell lines, but the level of fluorescence staining varied between the cell lines. In all lines tested, epratuzumab staining was much weaker than that observed with rituximab.

Because the rapid internalization of epratuzumab into target cells compromises the ability of the indirect assay to quantitate relative antigen expression, staining was also performed using directly labeled FITC-anti-CD20 (B1) and FITC-anti-CD22 (RFB4). CD22 expression is similar to or greater than CD20 expression in seven of the ten cell lines tested (Table 1). Three of the non-Burkitt cell lines (SU-DHL-6, RL, and DoHH2) express CD20 at much greater levels than the other seven cell lines. In these three lines, CD20 expression is substantially greater than CD22.

3.2. Effects of mAbs on proliferation of NHL cell lines

We utilized various approaches that present the mAbs to target cells in different formats, either in suspension or immobilized on polystyrene plates, to evaluate growth inhibition by epratuzumab and rituximab. In addition, the antiproliferative effects of the mAbs in suspension were examined with a second antibody for crosslinking, to mimic the role of receptor ligation *in vivo*, while that of the immobilized mAbs were evaluated with the cells stimulated by an anti-IgM antibody for crosslinking of cell surface IgM, as a means of activating B cells in lieu of BCR stimulation by antigen engagement.

As measured by the uptake of ^{3}H-thymidine, rituximab caused specific growth inhibition of the NHL cells, which was enhanced when the mAb was crosslinked, but the level of inhibition varied between cell lines with no apparent correlation with cell surface CD20 expression. SU-DHL-6 was markedly more sensitive to rituximab than other cell lines. In the absence of crosslinking, rituximab yielded approximately 88% inhibition of proliferation of SU-DHL-6 cells, which increased with crosslinking to 98% (data not shown). In Ramos and Daudi cells, greater than 60% inhibition was seen with crosslinked rituximab compared to less than 40% without crosslinking.

In contrast, the anti-CD22 mAb epratuzumab gave little or no inhibition of proliferation of the cell lines examined in this assay, and crosslinking with the anti-human IgG second antibody did not increase this effect. However, as shown in Fig. 1, the combination of rituximab and epratuzumab in the presence of crosslinking by second antibody was significantly more effective

Table 1
Antigen expression: flow cytometry assay (geometric mean fluorescence)

Cell line	Indirect staining			Direct staining		
	Control (hMN-14)	Rituximab	Epratuzumab	CD8	CD20	CD22
Burkitt						
Daudi	5.9	252.9	25.5	3.2	39.4	171.8
Raji	2.2	384.7	16.2	3.6	53.1	84.0
Ramos	1.1	119.5	5.0	3.1	41.5	67.7
Non-Burkitt						
DoHH2	4.8	45.6	4.8	4.3	328.2	61.0
Karpas422	8.3	12.2	8.2	3.6	14.0	15.3
Namalwa	2.5	7.3	6.8	n.d.[a]	n.d.	n.d.
RL	3.1	158.9	5.3	3.5	196.0	21.3
SU-DHL-4	2.5	46.8	5.9	3.1	10.9	26.9
SU-DHL-6	1.6	599.5	5.5	2.3	419.9	38.9
SU-DHL-10	2.5	35.7	6.7	3.4	10.3	19.7
WSU-FSCCL	3.0	36.4	6.0	3.0	5.1	8.0

[a] not determined.

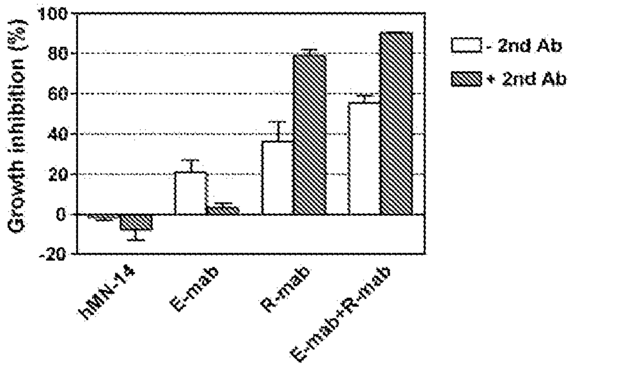

Fig. 1. Anti-proliferative effects of the combination of epratuzumab (E-mab) and rituximab (R-mab) by *in vitro* ^{3}H-thymidine uptake assay. Daudi cells were cultured with the mAbs (5 μg/ml) with or without a second antibody for crosslinking to mimic the role of effector cells *in vivo*. White bars, without second antibody; gray bars, with 20 μg/ml of F(ab′)$_2$ fragment of goat anti-human IgG (Fc) antibody. Error bars represent standard deviations of triplicates.

than crosslinked rituximab alone in inhibiting proliferation of Daudi cells (90.3 ± 0.4% versus 78.9 ± 3.0%, $P = 0.021$). Since the difference in anti-proliferative effect caused by the combination of epratuzumab and rituximab in the absence of crosslinking (55.6 ± 3.2%) compared to rituximab alone (36.3 ± 9.9%) did not reach statistical significance ($P = 0.065$), we conservatively conclude that under these conditions there is a trend suggesting that the combination of epratuzumab and rituximab is more effective than either alone.

3.3. *Effect of epratuzumab and rituximab on apoptosis*

The ability of epratuzumab and rituximab to induce apoptosis in NHL cells was measured in the presence of protein G for crosslinking using Annexin V/PI staining, followed by flow cytometry analysis. Fig. 2A and B, shows results of a representative experiment in Ramos, a sensitive cell line to rituximab-induced apoptosis. Rituximab incubation for 18 h in the presence of protein G resulted in 50% apoptosis, whereas no measurable apoptosis was induced by epratuzumab under these conditions. Combining epratuzumab with crosslinked rituximab does not significantly alter the levels of apoptosis obtained with crosslinked rituximab alone (Fig. 2C).

Induction of apoptosis was also evaluated by detection of hypodiploid DNA. Cells were cultured with the mAbs with or without second antibody, followed by DNA staining with PI. Cells were analyzed by flow cytometry, and positive fluorescence below the G1 region represents DNA fragmentation and is a measure of apoptosis. In all cell lines except SU-DHL-6, neither rituximab nor epratuzumab induced apoptosis in the absence of crosslinking (Table 2). In SU-DHL-6, the anti-CD20 mAb rituximab also induced apoptosis without crosslinking. Specific induction of apoptosis was seen with both rituximab and epratuzumab when an anti-IgG Fcγ crosslinking agent was used. The percent of hypodiploid nuclei induced by the two mAbs were similar, but relatively low (12–15%), in Daudi, RL, and Raji. SU-DHL-6 and Ramos cells were more sensitive to crosslinked-rituximab than the other cell lines (25.7%

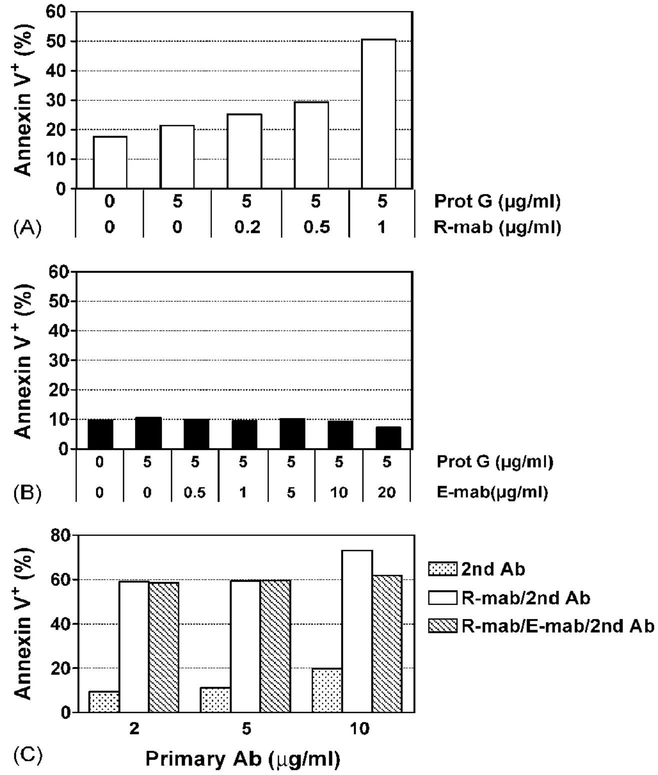

Fig. 2. Apoptotic effect of mAbs on Ramos determined by Annexin V. Ramos cells were plated at 0.5×10^6 cells/ml in medium containing varying doses of rituximab (R-mab) (A), epratuzumab (E-mab) (B), or both mAbs (C) and 5 μg/ml of the IgG crosslinking agent, protein G. Cultures were incubated for 24 h. Apoptosis was quantified by measuring percent of Annexin V-positive cells.

and 33.5% hypodiploid DNA, respectively), and both cell lines were also more sensitive to rituximab than to epratuzumab. It is interesting to note that Ramos exhibited the largest difference in sensitivity between rituximab and epratuzumab, with rituximab yielding almost two-fold the level of hypodiploid DNA as epratuzumab. Although technical differences between the Annexin V/PI staining and hypodiploid DNA measurement assays enabled detection of low levels of apoptotic induction by epratuzumab in Ramos only by the latter, the results of both

Table 2
Apoptotic effect of anti-CD20 MAbs as shown by PI staining (% hypodiploid DNA)

Cell line	2nd MAb	1st MAb			
		None	hMN-14	Rituximab	Epratuzumab
SU-DHL-6	None	4.2	3.9	9.4	4.4
	Anti-hIgG Fc	7.0	6.7	25.7	19.4
Daudi	None	1.4	1.3	1.2	1.1
	Anti-hIgG Fc	1.3	2.2	15.0	14.0
RL	None	2.4	1.8	3.7	1.8
	Anti-hIgG Fc	2.0	4.6	14.1	13.5
Ramos	None	4.1	2.3	2.5	1.7
	Anti-hIgG Fc	1.8	6.6	33.5	17.4
Raji	None	1.5	1.9	2.3	2.1
	Anti-hIgG Fc	2.0	5.3	12.1	12.8

studies are consistent in the demonstration that Ramos cells are more sensitive to rituximab than to epratuzumab. In contrast, detection of hypodiploid DNA after incubation with crosslinked-epratuzumab and crosslinked-rituximab were within 1% of each other in Daudi, RL, and Raji.

3.4. Amplification of BCR-induced cell death

An interesting and paradoxical effect of stimulating BCR is that it can result in proliferation of primary tonsillar B cells (Doody et al., 1995), but induces apoptosis when B-tumor cells are targeted (Chaouchi et al., 1995). It has also been reported that blocking CD22 translocation by immobilization of anti-CD22 antibody results in BCR-amplified activation of primary B cells (Doody et al., 1995). Epratuzumab was tested using a similar paradigm to determine its effect on primary tonsillar B cells and B-tumor cells when used in concert with stimulation of BCR. Culture plates were used as the solid support. Although epratuzumab can still bind cell surface CD22, its adsorption onto a plate should prevent CD22 internalization and restrict lateral diffusion of CD22 in the plane of the membrane.

Incubation of NHL cells with immobilized epratuzumab and rituximab in the presence of anti-IgM (in solution) yielded contrasting results to those obtained when the cells were treated with soluble mAbs. Fig. 3A shows the results obtained using Ramos cells as the target. Whereas crosslinked rituximab had a stronger inhibitory effect on cell growth than crosslinked epratuzumab when the cells and antibodies were studied in suspension, immobilized rituximab had no effect. Thus, rituximab treatment of Ramos cells did not yield a significant change in the rate of proliferation compared to the cells incubated in control IgG1-coated wells (not shown). Conversely, epratuzumab yielded a dose-dependent enhancement of the anti-proliferative effect in Ramos; approximately 20% in 1 μg/ml epratuzumab-coated wells ($P = 0.005$) and a greater than 50% increase in inhibition at 5 μg/ml and higher ($P < 0.0005$). This effect did not require the Fc portion of epratuzumab since $F(ab')_2$ fragment-coated plates yielded similar anti-IgM amplified cell death (not shown), indicating that the effect is mediated through epratuzumab binding to CD22 on the target cells. Since it is possible that the solid phase may mimic binding of epratuzumab to Fc-receptors of effector cells *in vivo*, we have not ruled out the possibility that the Fc fragment may be required for epratuzumab activity on cells, for example, within lymph nodes or lymphoma deposits. Consistent with the results described above with the ^{3}H-thymidine uptake assay, no effect on cell death above that observed with anti-IgM alone was seen when up to 50 μg/ml of epratuzumab was added in solution, even in presence of a crosslinking agent (data not shown).

The enhanced cell growth inhibition by epratuzumab was not as pronounced in all B-cell lines tested and there was no apparent correlation with cell surface CD22 expression (Fig. 3B). Although Daudi has the highest CD22 receptor density, it was insensitive to cell death amplification. The same was true for the

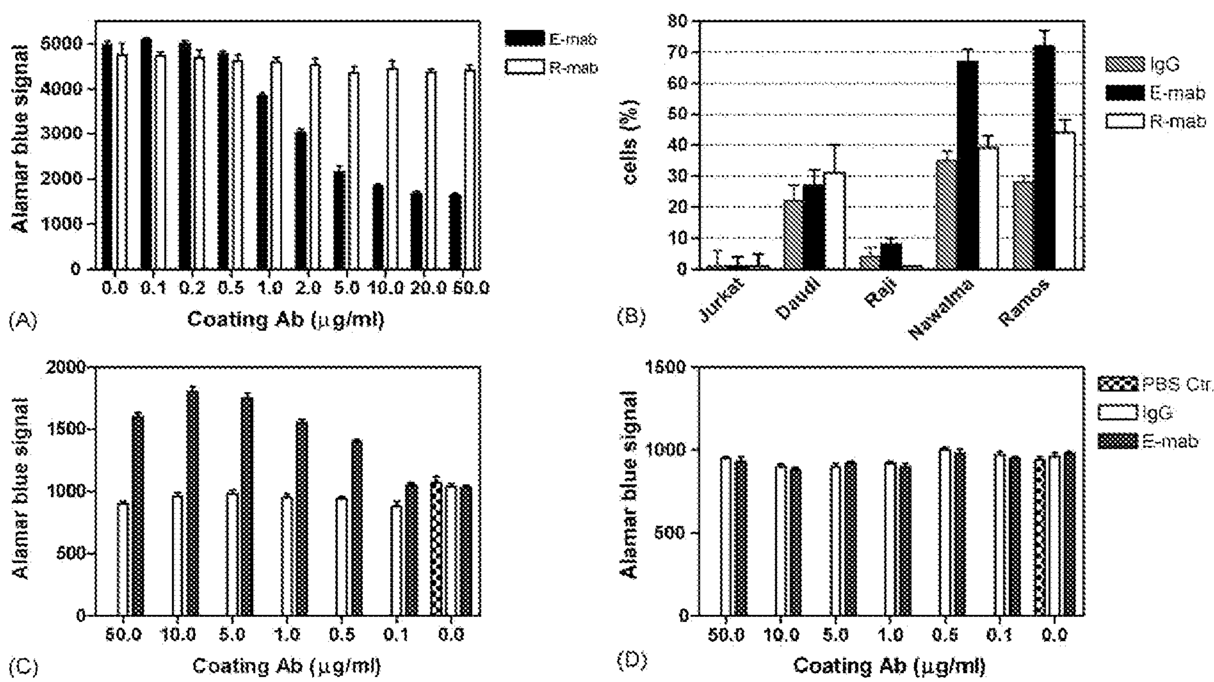

Fig. 3. Immobilized epratuzumab but not rituximab amplifies BCR stimulation-induced effects. NHL cell lines or fresh tonsillar B cells were plated on antibody-coated 96-well plates and stimulated with an anti-IgM crosslinking antibody. Viable cell population was measured after 48 h using Alamar Blue. (A) Comparison of the effect of immobilized epratuzumab and rituximab, on Ramos cells. Cells were stimulated with 5 μg/ml anti-IgM antibody. (B) Anti-IgM-mediated death in relation to CD22 density. B-lymphoma cell lines were plated on antibody-coated tissue culture plate (10 μg/ml), and stimulated with an anti-IgM antibody, 5 μg/ml. Jurkat cells (CD22- and CD20-negative cell line) were used as a negative control. Percent cell death is calculated using the cells not treated with anti-IgM as 100% viable cells, and well containing no cells as 100% cell death. (C) Fresh tonsils were dissociated and isolated B cells plated on immobilized epratuzumab or IgG1 control antibody in the presence of 5 μg/ml anti-IgM antibody. (D) Fresh tonsil B cells were incubated with epratuzumab or IgG1 control antibody in solution in the presence of 5 μg/ml anti-IgM. Viable cell population was measured after 48 h using an Alamar Blue assay.

Raji cell line, which has a CD22 receptor density comparable to that of Ramos cells. Both Namalwa and Ramos cells showed the same sensitivity to being plated on epratuzumab-coated plates. It is possible that cell surface IgM density may be related to this BCR-activation amplification, since we found IgM expression to be 3.4-fold higher in Ramos than Daudi cells (data not shown).

Fig. 3C and D, shows the effects of epratuzumab on primary human tonsillar B cells. Primary B cells plated on immobilized epratuzumab have enhanced proliferation in response to anti-IgM stimulation, compared to cells plated on a control human IgG1 mAb (Fig. 3C) or rituximab (not shown). The epratuzumab effect shows a dose-dependency, saturating at a coating concentration of approximately 5 μg/ml. However, when epratuzumab was added in solution no amplification was observed at any of the concentrations tested (Fig. 3D). These results suggest that preventing CD22 translocation (i.e., internalization or lateral movement), rather than down-regulation of the quantity of CD22 on the cell surface, mediates this effect.

3.5. *Inhibition of cell proliferation by immobilized epratuzumab independent of BCR activation*

The effects of mAbs on inhibiting cell proliferation and inducing apoptosis are dependent on a number of factors including culture conditions. This is due to competition of the added anti-B-cell mAbs with the components in the culture medium for promoting cell growth and protection from apoptosis. The antiproliferative effects of immobilized epratuzumab and rituximab were evaluated on cells stressed by lowering the concentration of FBS in the culture medium from 10% to 3%. Ramos and D1-1, a subclone of Daudi, were used. As shown in Fig. 4, both Ramos and D1-1 cell proliferation were affected by immobilized epratuzumab in the absence of added anti-IgM. Three days of culturing in epratuzumab-coated wells resulted in ~30% ($P = 0.003$) and >50% ($P = 0.001$) inhibition of proliferation of these cell lines. Under the same conditions, immobilized rituximab and isotype-matched control IgG1 mAbs, hLL1 (B-cell binding, anti-CD74) and hMN14 (non-B-cell binding), did not have significant effects. Statistically significant differences between epratuzumab and rituximab were observed ($P = 0.009$ for both cell lines).

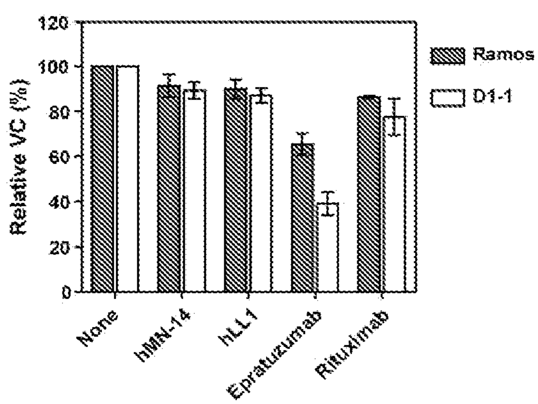

Fig. 4. Inhibition of cell growth by immobilized mAbs. Ramos and D1-1 cells were cultured in 48-well cell culture plates pre-coated with 5 μg/ml mAb, as indicated. Medium containing ~3% FBS was used in these experiments. After 3 days of incubation in a CO_2 incubator, 37 °C, the viable cell numbers were determined by MTT assay. The results shown are the average of three sets of experiments.

3.6. *Effect of epratuzumab and rituximab on CDC and ADCC*

CDC was measured on three B-cell lines, Ramos, Daudi, and Raji. Rituximab was shown to cause lysis in all three cell lines, while epratuzumab had essentially no effect. Daudi was the most sensitive of the B-cell lines tested, with 100% lysis at 1 μg/ml rituximab (Fig. 5A), while Raji and Ramos cells required 10 μg/ml rituximab to achieve the same effect (data not shown).

Daudi was used to examine whether epratuzumab competes with rituximab for recruiting complement and if it affects rituximab's cytolytic activity when the two mAbs are combined. Jurkat T-cell lymphoma was used as a negative control. When increasing concentrations of epratuzumab were added along with 1 μg/ml rituximab, no reduction in CDC was observed (Fig. 5B). This suggests that although epratuzumab does not mediate complement-dependent cell lysis, it would not interfere with rituximab's cytolytic activity when these two mAbs are combined in an immunotherapy setting. In other experiments, we pre-treated B cells with saturating amounts of epratuzumab for 1 h, overnight, or 65 h prior to adding rituximab and complement. This pre-incubation step did not modulate rituximab's CDC activity (data not shown).

The ability of epratuzumab to induce ADCC was evaluated, comparing it to and combining it with rituximab, using Raji as the target cells. Rituximab induced up to 40% ADCC in the presence of effector cells from donor 1400 (Fig. 5C). Epratuzumab showed more moderate, but significant activity ($P < 0.0002$ at E:T ratios 1:50 and 1:25, $P = 0.004$ at E:T ratio 1:100, compared to IgG1 control) in the same experiment. The ADCC assay was repeated with seven different donors to allow screening through allelic polymorphism existing in the FcγRIII population with similar findings (Fig. 5D). Similar results were also observed when ADCC was evaluated in Daudi and SU-DHL-6 (Fig. 5E and F). The combination of the two antibodies did not result in significantly increased efficacy. Thus, rituximab was consistently more potent than epratuzumab in mediating ADCC. However, adding epratuzumab to rituximab had no detrimental effect on rituximab-mediated ADCC.

4. Discussion

CD22, a B-cell restricted transmembrane sialo-glycoprotein, has recently been shown to play a complex role in the regulation of normal B-cell function, both as an adhesion molecule and as a component of the B-cell activation complex (Nitschke, 2005; Tedder et al., 2005). Its role as a lectin-like adhesion receptor is facilitated through CD22 binding of α2,6-linked sialic acid-bearing ligands. Two extracellular (N-terminal) domains have been shown to be required for ligand binding (Engel et al., 1995). The role of CD22 in modulating signaling through the BCR complex is due to phosphorylation of three immunoreceptor

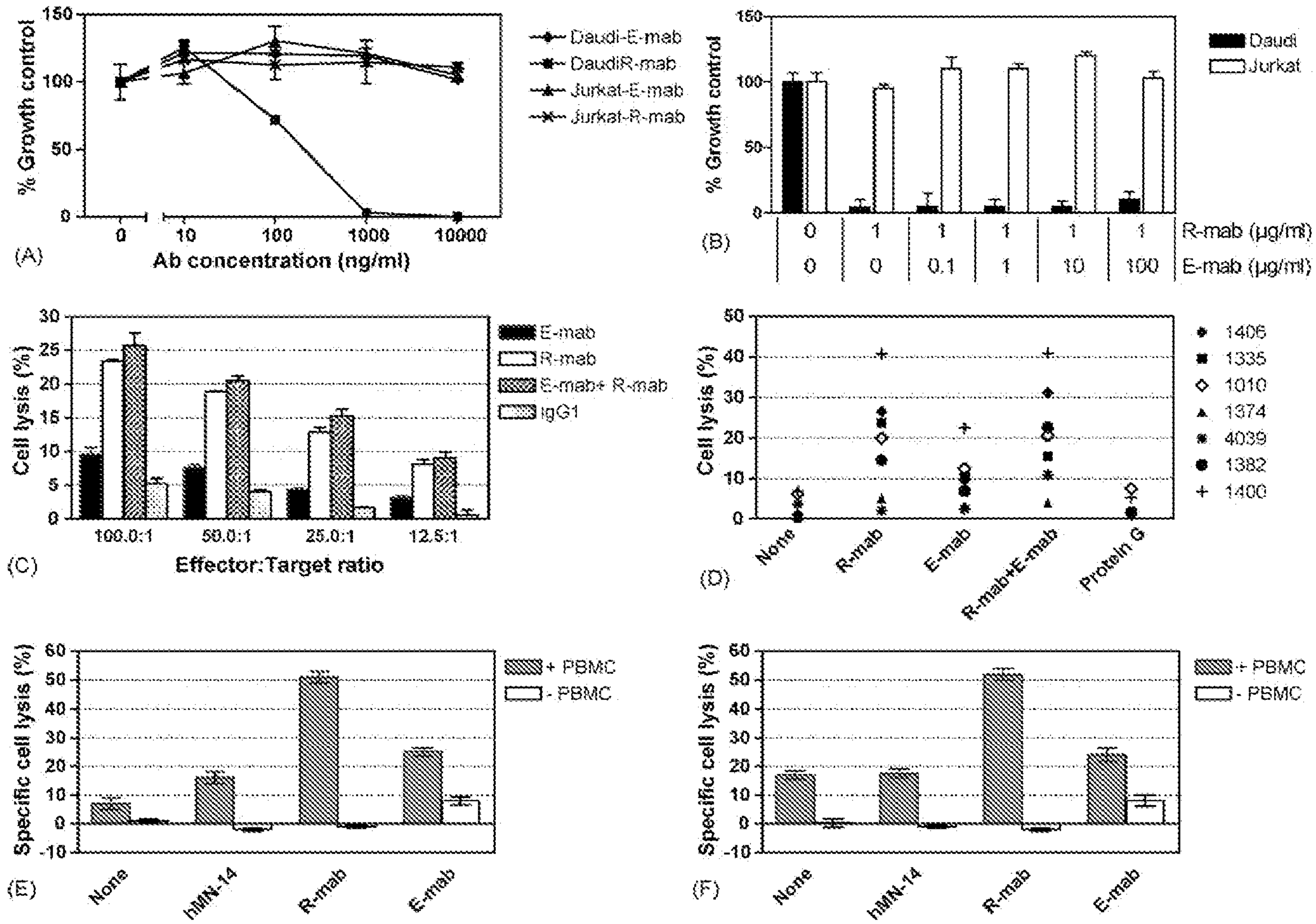

Fig. 5. Effect of rituximab and epratuzumab on CDC and ADCC. (A) Daudi cells or control cells (Jurkat) were treated with either rituximab (R-mab) or epratuzumab (E-mab) at the concentrations shown in the presence of complement. Cell viability was measured using Alamar Blue and reported as % of viable population relative to cells treated with complement only (no mAb). (B) Daudi or control cells (Jurkat) were treated with a fixed dose of rituximab (1 μg/ml) together with increasing concentrations of epratuzumab. The lytic effect of rituximab on Daudi was approximately 100% and unchanged through the tested conditions. ^{51}Cr-labeled NHL cells were incubated with anti-B-cell mAbs in the presence of human peripheral blood mononuclear cells for measurement of ADCC induction by epratuzumab and rituximab. (C) ADCC in Raji cells was assessed across a range of effector cell to target cell ratios using epratuzumab and rituximab singly and in combination. (D) Summary of ADCC activity for epratuzumab, rituximab, or both using effector cells from a panel of healthy donors. (E) ADCC in Daudi cells. (F) ADCC in SU-DHL-6 cells.

tyrosine-based inhibitory motifs (ITIM) on its intracellular tail upon BCR stimulation. Phosphorylated CD22 can then recruit tyrosine phosphatase SHP-1 and other effector molecules, which in turn limit BCR signaling (Nitschke, 2005; Tedder et al., 2005). Thus, a major role of CD22 is as a negative regulatory molecule limiting the intensity of BCR-generated signals. The functional relationship between the ligand-binding domains and the signaling domains has been an area of active investigation. Hypotheses have included direct mediation of CD22-BCR interactions by sialic acid binding (Cyster and Goodnow, 1997; Kelm et al., 2002), and the ability of α2,6-linked sialic acid carrying proteins to sequester CD22 away from the BCR (Doody et al., 1995).

In this report, we engaged several different cell-based assays to evaluate the effects of the anti-CD22 mAb epratuzumab on B-lymphoma cells to gain an understanding of these effects in light of the biology of CD22. Moreover, these assays explored whether a rationale exists for combining epratuzumab with rituximab clinically. A number of anti-CD22 mAbs that bind to different CD22 epitopes have been reported. Many of these bind to the two N-terminal Ig-like domains essential for CD22-mediated adhesion, and fully or partially block CD22-mediated adhesion. MAbs in this group include the HB22 series, as well as others (Engel et al., 1993). Epratuzumab and the anti-CD22 mAb, RFB4, bind to the third domain (epitope B) (Stein et al., 1993). Because CD22 has been reported to regulate B-cell function through both ligand-dependent and ligand-independent mechanisms, it is likely that treatment with these various mAbs may result in a range of clinical outcomes.

The activity of epratuzumab was first evaluated as a soluble agent either alone or in a crosslinked form. Crosslinking was performed with anti-IgG or protein G, agents which can be considered to mimic the receptor ligation that may occur in the natural setting. In solution, epratuzumab was not found to be an effective cytotoxic agent, either alone or crosslinked. This contrasts with the effectiveness of crosslinked rituximab. The efficacy of crosslinking rituximab has been noted previously, and extensively evaluated (Mathas et al., 2000; Shan et al.,

1998; Zhang et al., 2005). Our results are consistent with these earlier observations. In all B-cell lines studied, we observed specific inhibition with crosslinked rituximab, with significantly lower anti-proliferative effects in the absence of a crosslinking agent. It has been hypothesized that a substantial proportion of the cytotoxic effects of anti-CD20 mAbs observed in clinical trials may be due to an analogous mechanism; namely, *in vivo* ligation of malignant B cells by anti-CD20 mAbs followed by FcR-mediated crosslinking by macrophages or other accessory molecules (Gong et al., 2005; Shan et al., 1998). Hyper-crosslinking of rituximab redistributes CD20 into lipid rafts followed by activation of Src-family tyrosine kinases such as *Lyn*, *Fyn*, and *Lyc* (Deans et al., 1995) kinases, which are also involved in the modulation of signaling events after BCR activation (Kurosaki, 1998). Our observation that the combination of rituximab and epratuzumab was significantly more effective than rituximab alone in inhibiting proliferation of Daudi cells (in the presence of crosslinking by second antibody) may therefore be due to the simultaneous activation of these converging mechanisms.

In contrast to the lack of *in vitro* activity of soluble epratuzumab, we found that immobilized epratuzumab was able to yield a significant reduction in viable cell count in two NHL cell lines, Ramos and Namalwa, incubated with anti-IgM, as well as a significant increase in the proliferation of tonsillar B cells. Immobilization on plastic was used to prevent the antibody and its target antigen from being internalized after they interact on the cell surface, and to restrict lateral diffusion of antigens in the plane of the membrane. Doody et al. (1995) suggested that incubation of $CD22^+$ cells with immobilized anti-CD22 sequesters CD22 from surface IgM, thereby disabling the inhibitory feedback effect CD22 would otherwise exert on the activation of the B cell via its interaction with BCR. Therefore, restricting lateral diffusion of CD22 in the plane of the membrane (by incubating with an immobilized anti-CD22 mAb) enhances the response of B cells to the ligation of surface IgM. The physiologically relevant analog of immobilized antibody to CD22 may be the B and T cells surrounding antigen-stimulated B cells in secondary lymphoid organs. Immobilized epratuzumab was also cytotoxic to NHL cell lines without anti-IgM when the cells were stressed by culturing in low serum concentration. Thus, a second mechanism must be involved. It is possible that disturbing the cellular distribution of CD22 on the B-cell membrane or preventing internalization of the CD22-epratuzumab complex may allow any signaling caused by direct interaction between the antibody and antigen (not necessarily involving the BCR) to be potentiated. Interactions with other ligands for CD22 may be involved, possibly CD45, a known ligand of CD22 (Sgroi et al., 1995).

The effects of anti-CD22 mAbs on B-cell function have been studied by multiple groups with apparently conflicting results. CD22 ligation was reported to generate stimulatory signals in some assay systems (Doody et al., 1995; Pezzutto et al., 1987, 1988) and apoptotic signals in others (Chaouchi et al., 1995; Tuscano et al., 1999). In our assay system, immobilized epratuzumab plus anti-IgM had differing effects dependent on the cell type. The function of CD22 in the regulation of B-cell survival and signal transduction is clearly complex, and thus it is not surprising that varying responses have been observed experimentally when different assay systems and different cell types, varying in levels of maturation and activation, are used. Indeed, antigen receptor signaling is known to have different consequences depending on the stage of B-cell maturation and the strength and duration of the signal, variously triggering either cell proliferation, anergy, or death by apoptosis (Chaouchi et al., 1995; Cyster and Goodnow, 1997).

Our observation that rituximab induces CDC and ADCC in B-lymphoma cell lines is consistent with previous reports on rituximab's induction of CDC (Flieger et al., 2000; Reff et al., 2002) and ADCC (Flieger et al., 2000; Reff et al., 1994). However, unlike rituximab, no complement-mediated lysis could be detected following incubation with epratuzumab. Moderate, but significant, ADCC activity was observed when NHL cells were treated with epratuzumab, supporting the findings reported previously by Gada et al. (2002). It is interesting to note that the humanized anti-CD20 mAb, hA20 (IMMU-106), was constructed using the same human V framework and $\gamma 1/\kappa$ constant region sequences as epratuzumab, yet hA20 induces CDC at similar levels as rituximab (Stein et al., 2004). We therefore conclude that differences in antigen specificity rather than a deficiency in physical sequences or the tertiary structure of epratuzumab limit its activity in these assays. It is possible that the lack of epratuzumab-induced CDC may be due in part to the distance between the epitope to which epratuzumab binds and the plasma membrane, resulting in lack of activation of the complement cascade. Alternatively, the lack of CDC activity and modest ADCC activity of epratuzumab may be due to its rapid internalization following antigen binding, resulting in an apparently reduced cell surface expression of CD22 (Carnahan et al., 2003). In the case of rituximab, induction of CDC has been demonstrated to correlate with the level of CD20 expression in B lymphomas (Manches et al., 2003) and normal circulating B cells (Vugmeyster et al., 2003). The ability of rituximab and other anti-CD20 mAbs to induce CDC also correlates with their ability to translocate CD20 into lipid rafts (Cragg et al., 2003). The relationship between CD22, anti-CD22 mAbs, and lipid rafts remains to be established, but may also factor into the inability of epratuzumab to induce CDC. Indeed, it has been reported that CD22 is excluded from lipid rafts (Pierce, 2002). Although the ability of rituximab to induce CDC has been credited with a large part of its clinical efficacy, it has also been correlated with the occurrence of severe first-dose side effects of rituximab treatment (van der Kolk et al., 2001). Since combining rituximab and epratuzumab *in vitro* did not decrease rituximab's induction of CDC or ADCC, the combined use of these two mAbs may yield increased therapeutic benefit without adding to this toxicity. Indeed, the full-dose combination of epratuzumab with rituximab was well tolerated and had significant clinical activity in a recent study of 23 patients with recurrent B-cell lymphoma (Leonard et al., 2005). In addition, potentiation of anti-CD20 mAb activity has been observed in *in vivo* animal studies when epratuzumab was combined with rituximab or hA20 (Hernandez-Ilizaliturri et al., 2002; Stein et al., 2004).

In summary, comparative studies using several different cell-based assays demonstrated that epratuzumab has distinct effects on cell growth from rituximab. These effects are consistent with the biology of its target, CD22, an immune regulatory receptor. In the soluble form, rituximab is more potent in inhibiting proliferation when crosslinked, while epratuzumab is not effective as a soluble agent in either a crosslinked or non-crosslinked form. However, epratuzumab, but not rituximab, is able to inhibit cell proliferation when immobilized. Unlike rituximab, no complement lysis could be detected, and ADCC levels were modest when NHL cells were treated with epratuzumab. Combining rituximab and epratuzumab *in vitro* did not decrease rituximab's acute and potent effects in inducing apoptosis, CDC, and ADCC. In fact, *in vitro* studies on the combination of rituximab and epratuzumab indicated that the combination may be more effective than rituximab alone in inhibiting proliferation of Daudi cells. Costimulatory effects of anti-CD20 and -CD22 have been reported previously; Pezzutto et al. (1987) noted that when the anti-CD22 mAb, HD6, and the anti-CD20 mAb, 1F5, were combined, a higher level of proliferation was induced in resting B cells than with either mAb alone.

The murine-human chimeric antibody, rituximab, has shown marked success in the treatment of NHL. However, there is still a substantial percentage of patients who do not respond (∼50%) (Davis et al., 2000). Thus, there is an ongoing effort to improve these results. We examined the similarities and differences of epratuzumab and rituximab to better understand the manner in which they exert their cytotoxic effects, and whether they may be effectively combined for therapeutic benefit. We conclude that as a single and naked antibody, epratuzumab's mode of action results mostly from its ability to enhance the response of tumor cells to antigen activation of BCR, a function consistent with the ability of epratuzumab to induce CD22 phosphorylation. This activity would presumably occur mostly in lymph nodes and other compartments where cell density and geometry allows for epratuzumab binding both through its Fc portion and to its target, CD22. We postulate that dendritic cells or macrophages bearing the FcγRIII receptor may ligate or cluster CD22 through epratuzumab on targeted tumor cells or on a subpopulation of B cells implicated in autoimmunity. Upon antigenic stimulation, these cells may be more susceptible to programmed cell death compared to non-antibody-treated cells in this compartment. In circulation, as we have shown previously, it is likely epratuzumab induces a rapid and prolonged internalization of CD22, perhaps also impairing the homing of the tumor cells to bone marrow (Nitschke et al., 1999). Clinically, epratuzumab has induced remission of disease in NHL (Leonard et al., 2003), and in systemic lupus (Kaufmann et al., 2004) and Sjögren's syndrome (Steinfeld et al., 2005), as well as a depletion of circulating B cells, but not as severe as reported for rituximab (Reff et al., 1994).

Our *in vitro* studies showed rituximab to be a compatible co-treatment with epratuzumab, because its mode of action is very distinct and unaffected by combination with epratuzumab. These observations suggest the possibility that in patients epratuzumab and rituximab may have non-overlapping, complementary, and beneficial efficacy, thus warranting assessment of this hypothesis in clinical trials. Indeed, initial single- and multiple-center trials of this combination of antibodies in indolent and aggressive NHL have suggested a therapeutic advantage for the combination without increased host toxicity (Leonard et al., 2005; Strauss et al., 2005).

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ORIGINAL REPORT

Combination Antibody Therapy With Epratuzumab and Rituximab in Relapsed or Refractory Non-Hodgkin's Lymphoma

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A B S T R A C T

Purpose
To explore the safety and therapeutic activity of combination anti–B-cell monoclonal antibody therapy in non-Hodgkin's lymphoma (NHL).

Patients and Methods
Twenty-three patients with recurrent B-cell lymphoma received anti-CD22 epratuzumab 360 mg/m^2 and anti-CD20 rituximab 375 mg/m^2 monoclonal antibodies weekly for four doses each. Sixteen patients had indolent histologies (15 with follicular lymphoma) and seven had aggressive NHL (all diffuse large B-cell lymphoma [DLBCL]). Indolent patients had received a median of one (range, one to six) prior treatment, with 31% refractory to their last therapy and 81% with high-risk Follicular Lymphoma International Prognostic Index scores. Patients with DLBCL had a median of three (range, one to eight) prior regimens (14% resistant to last treatment) and 71% had high intermediate–risk or high-risk International Prognostic Index scores. All patients were rituximab naïve.

Results
Treatment was well tolerated, with toxicities principally infusion-related and predominantly grade 1 or 2. Ten (67%) patients with follicular NHL achieved an objective response (OR), including nine of 15 (60%) with complete responses (CRs and unconfirmed CRs). Four of six assessable patients (67%) with DLBCL achieved an OR, including three (50%) CRs. Median time to progression for all indolent NHL patients was 17.8 months.

Conclusion
The full-dose combination of epratuzumab with rituximab was well tolerated and had significant clinical activity in NHL, suggesting that this combination should be tested in comparison with single-agent treatment.

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INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of lymphoid neoplasms, with diverse molecular characteristics, biology, clinical presentation, response to treatment, and survival.[1-4] Despite the high response rate of indolent NHL to initial therapy, subsequent therapeutic interventions generally are progressively less effective in controlling disease, and cure is not usually expected.[5,6] Diffuse large-cell NHL can be cured with first-line combination chemotherapy, but nearly 50% of patients will have progressive disease after primary therapy, requiring subsequent treatment that is associated with substantially lower cure rates.[5-9]

Rituximab (Rituxan; Genentech Inc, South San Francisco, CA; and Biogen Idec Pharmaceuticals, San Diego, CA), the first monoclonal antibody (MAb) approved for

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the treatment of CD20-positive B-cell NHL in 1997, is a human-mouse chimeric immunoglobulin G1 (IgG1) MAb.[10] It targets the CD20 antigen present on B cells and produces rapid and severe B-cell depletion. Although rituximab is useful as a monotherapy and in combination with chemotherapy for various forms of NHL, virtually all patients experience disease relapse after single-agent treatment.[10] Given that another B-cell antigen, CD22, is also abundantly expressed in a similar pattern and frequency as CD20 in B-cell NHL,[11] we examined a humanized anti-CD22 MAb, epratuzumab, in dose-escalation trials of NHL patients with indolent and aggressive disease.[12,13]

The encouraging safety and efficacy results obtained as a monotherapy given once weekly during 4 weeks stimulated our interest in examining whether administration of full doses of epratuzumab combined with rituximab would be well tolerated and could perhaps enhance responses compared with the known experience with either antibody alone. This logical next step in the development of immunotherapy of NHL explores the concept that agents targeting two different antigen sites on cancer cells, which may at least in part have different mechanisms of action, might have additive or synergistic antilymphoma effects or could potentially overcome single-agent resistance. In this phase II study, we report the first results in patients with relapsed/refractory indolent and aggressive NHL receiving a combination of two anti–B-cell antibodies, epratuzumab and rituximab.

PATIENTS AND METHODS

Study Design

This was a phase II single-center trial evaluating four intravenous infusions of the chimeric anti-CD20 MAb, rituximab, combined with epratuzumab, a humanized anti-CD22 antibody, in patients with relapsed or refractory indolent or aggressive NHL. The objectives were to evaluate the tolerability, safety, dose-limiting toxicities, immunogenicity, and clinical activity of the combined antibody immunotherapy. Initially, seven patients received the first dose of epratuzumab on the first day, followed by rituximab 2 days later. After the safety of this combination was confirmed, subsequent patients received epratuzumab followed within 1 hour by rituximab, infused during 4 to 6 hours on the same day. Infusions of both agents were administered weekly for 4 consecutive weeks, with assessments for response and toxicity performed 4 weeks after the last infusion. The patients were reevaluated every 3 to 4 months for the first 2 years, and every 6 months thereafter until disease progression.

Antibodies

Epratuzumab (humanized IgG1 [κ], anti-CD22 monoclonal antibody [hLL2]) was produced and subjected to quality control at Immunomedics Inc (Morris Plains, NJ), and was administered at dose of 360 mg/m^2/wk. Rituximab (chimeric IgG1 [κ], anti-CD20 monoclonal antibody) was administered at a dose of 375 mg/m^2/wk for 4 consecutive weeks; each infusion occurred after the administration of epratuzumab, except in the first cohort of patients (as described above).

Study Population

Patients at least 18 years of age with histologically confirmed, relapsed, or refractory indolent and aggressive B-cell NHL were enrolled, provided they experienced disease progression after at least one prior therapy regimen, had a performance status of less than 2 according to the Eastern Cooperative Oncology Group criteria, were not pregnant or lactating, and had a life expectancy of more than 3 months. The patients were required to have CD20$^+$ and CD22$^+$ NHL, as determined by either immunohistochemistry or flow cytometry of tumor specimens obtained at any time before enrollment. The WHO classification of lymphoma subtypes was used for this report.[14,15] Within 4 weeks before receiving study treatment, patients were required to have a hemoglobin more than 8.0 g/dL, an absolute granulocyte count $\geq$ 1,500/μL, a platelet count $\geq$ 75,000/μL, a serum creatinine of $\leq$ 1.5$\times$ the upper limit of normal, a serum bilirubin $\leq$ 1.5$\times$ the upper limit of normal, absence of positive hepatitis B and C serology, and nonbulky disease $\leq$ 10 cm in the largest diameter. They were required to be at least 4 weeks beyond any chemotherapy, radiotherapy, or biologic therapy, and 2 weeks beyond corticosteroid use. All patients were rituximab naïve. Patients with CNS disease, HIV, or Richter's lymphoma were not eligible. The protocol was approved by the Weill Medical College of Cornell University–New York Presbyterian Hospital Institutional Review Board, and written informed consent was obtained from all patients.

Study End Points

Safety end points included the incidence of adverse events (AEs), including those occurring during or within 7 days of the MAb infusions and all later events deemed possibly or probably related to treatment, and change in the human antihuman antibody (HAHA) status, laboratory values, and infusion-day vital signs. AEs were recorded throughout the study and graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Monitoring included frequent hematology and serum chemistry profiles, periodic monitoring of immunoglobulins, and immunophenotyping of blood mononuclear cells. The patients were screened for serum anti-CD22 antibody (HAHA) using an enzyme-linked immunosorbent assay (Immunomedics Inc), which has a sensitivity of 5 ng/mL. Samples were obtained within 4 weeks before study entry, 24 hours after the last infusion, at restaging, and 3 months after the last treatment.

Efficacy

Responses were assessed according to the International Workshop NHL response criteria.[16] Bidimensional computed tomography data for the neck/chest and abdomen/pelvis were collected at screening, restaging, and follow-up visits for evaluation of disease response. Bone marrow aspirates and biopsies were performed at study entry and as required to confirm complete responses (CRs). Time to progression (TTP) was measured from the first infusion until the first evidence of progression.

Statistical Analyses

All patients who received more than one dose of epratuzumab and rituximab were included in the safety and efficacy analyses. The analyses of the objective response (OR) rate for indolent and aggressive NHL and TTP were based on the assessable patient subset (all enrolled patients who received > one dose of epratuzumab and rituximab, and had $\geq$ one post-treatment

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evaluation for response or had withdrawn from the study before the first post-treatment evaluation of response because of disease progression). Duration of response was analyzed for the patients who responded. Median duration of response and TTP were estimated using the Kaplan-Meier method.[17]

RESULTS

Patients

Between September 2000 and July 2002, 23 patients with indolent (n = 16) and aggressive (n = 7) NHL who received at least one dose of epratuzumab and rituximab were enrolled onto the study and included in the evaluation of safety. Twenty-two of these patients were included in the evaluation of efficacy; one patient withdrew consent after only one infusion of epratuzumab and rituximab because of pre-existing disease-related symptoms (pain), and was not included in the evaluation of efficacy because of inadequate post-treatment evaluation. Demographic and clinical characteristics for enrolled patients are listed in Table 1. Fifteen patients with follicular NHL, one patient with marginal-zone NHL, and seven patients with diffuse large B-cell lymphoma (DLBCL) were enrolled. Patients with indolent and

Table 1. Baseline Demographic and Clinical Characteristics for Patients Receiving at Least One Dose of Epratuzumab

Baseline Characteristic	Indolent Patients (n = 16) No. of Patients	Indolent Patients (n = 16) %	Aggressive Patients (n = 7) No. of Patients	Aggressive Patients (n = 7) %
Age, years				
Median	64.5		67	
Range	31-84		26-86	
Females	10	63	4	57
No. of prior therapies				
Median	1		3	
Range	1-6		1-8	
Prior rituximab treatment	0		0	
Prior high-dose chemotherapy and stem-cell transplantation (%)	0		1	14
Response to last prior treatment	11	69	6	86
Time from end of last treatment, years				
Median	2.5		1	
Range	0.1-10.6		0-10.4	
Time from initial diagnosis, years				
Median	4.55		2.5	
Range	0.1-22.2		1-12.4	
WHO classification				
Diffuse, large B cell	—		7	100
Follicular grade I	4	25	—	
Follicular, grade II	11	69	—	
Marginal zone B-cell	1	6	—	
Stage of NHL: No. (%)				
III	8	50	4	57
IV	8	50	3	43
Bone marrow involvement	5	31	2	29
ECOG performance score = 0	4	25	1	14
Bulky disease at least 5 cm	9	56	3*	43
SPD, cm^2				
Median	50.9*		22.1*	
Range	6.42-284		3.42-266	
LDH > normal	7	44	5	71
IPI				
1	3	19	1	14
2	9	56	1	14
3	4	25	4	57
4	—		1	14
Peripheral-blood B-cell counts, CD20				
Median	13		17	
Range	< 1-65		3-26	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; SPD, sum of products of bidimensional measurements; LDH, lactate dehydrogenase; IPI, International Prognostic Index.
*Unknown for one patient.

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aggressive NHL had similar demographic and clinical characteristics. Slightly more females than males were enrolled (63% with indolent and 57% with aggressive NHL, respectively). Additional characteristics included the following: 100% were stage III/IV and approximately one third had bone marrow involvement at study entry. Patients with indolent NHL had a median of 4.5 years from diagnosis, and patients with aggressive NHL had a median of 2.5 years from diagnosis. The median age was 64.5 years for indolent NHL patients (range, 31 to 84 years) and 67 years for DLBCL patients (range, 26 to 86 years). All patients had at least one prior therapy; there was a median of one prior therapy (range, one to six prior therapies) for indolent NHL patients and of three prior therapies (range, one to eight prior therapies) for aggressive NHL patients. No patient had received prior therapy with rituximab.

Safety

Treatment was well tolerated, and no dose-limiting toxicity was encountered. After seven patients received their week 1 doses separated by 2 days (treatment was received on days 1 and 3) without difficulty, subsequent patients received therapy with both epratuzumab and rituximab on days 1, 8, 15, and 22. Toxicities encountered during the course of treatment are summarized in Table 2. Most clinical AEs (91%) were mild to moderate (grade 1 or 2) and self-limited. The majority of patients experienced AEs during the first infusion, and both the incidence and frequency declined with subsequent infusions. Approximately 61% (14 patients) had AEs that were considered to be related (probably or possibly related, or of unknown relationship) to the study treatment. All AEs with at least a 10% frequency are reported in Table 2. Given the close temporal relationship of the administration of the agents, it is not possible to attribute AEs definitively to one antibody or the other. No clinically significant changes in laboratory measurements (including hematology values and serum chemistries or vital signs) were noted, and no serious treatment-related AEs occurred. No patient developed HAHA against epratuzumab.

Rituximab in combination with epratuzumab ablated the blood B-cell levels for up to 6 months, as measured by $CD19^{+}$ and $CD20^{+}$ cell counts, but mean/median IgM, IgA, and IgG levels remained at baseline levels at all evaluations.

Responses to Treatment

In the 16 assessable patients with indolent NHL, the OR rate was 63% (10 of 16 patients), with nine patients (56%) achieving a CR or unconfirmed CR (CRu) and one patient achieving a partial response (PR), as listed in Figure 1. We also analyzed the CR rate of the indolent NHL population according to the follicular lymphoma international prognostic index score (FLIPI) at study entry.[6] The FLIPI is currently the most widely accepted prognostic assessment for indolent NHL. Of 16 indolent NHL patients, two of three patients with an intermediate-risk FLIPI score achieved a CR, whereas five of 13 patients (46%) with a high-risk FLIPI score achieved a CR or CRu. In the six assessable patients with DLBCL, the OR rate was 67% (four of six patients), with three CRs and one PR. An intent-to-treat analysis (including one patient who was considered inassessable because only one infusion was administered) results in an OR rate of 57% for the DLBCL group. The median time to response was 57 days. The median duration of remission for indolent and aggressive NHL has not been reached. Median TTP for indolent NHL was 17.8 months. The Kaplan-Meier curves of duration of remission and TTP are shown in Figures 2 and 3. Responses are ongoing in seven of the 16 patients with indolent NHL and three of the six patients with aggressive DLBCL.

Table 2. All Adverse Events With at Least 10% Incidence

Preferred Term	No. of Patients (n = 23)	%
Pyrexia	7	30
Rigors	7	30
Fatigue	5	22
Influenza-like illness	3	13
Pain NOS	3	13
Arthralgia	4	17
Pain in limb	3	13
Nausea	3	13
Headache NOS	4	17
Flushing	3	13

Abbreviation: NOS, not otherwise specified.

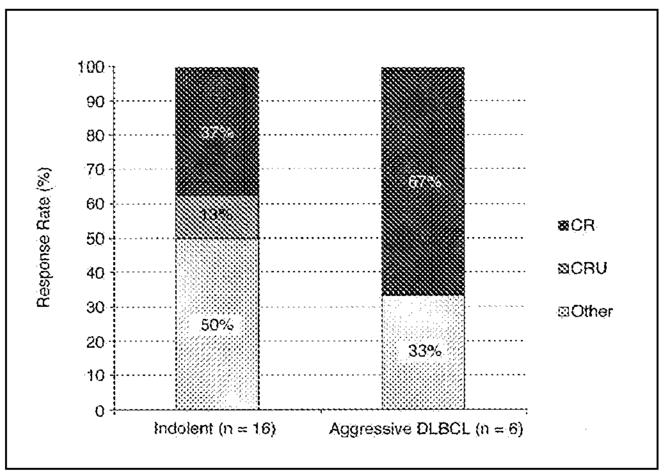

Fig 1. Response rate for patients with indolent-follicular, indolent-other, and all indolent NHL, and aggressive diffuse large B-cell lymphoma. CR, complete response; CRU, unconfirmed CR.

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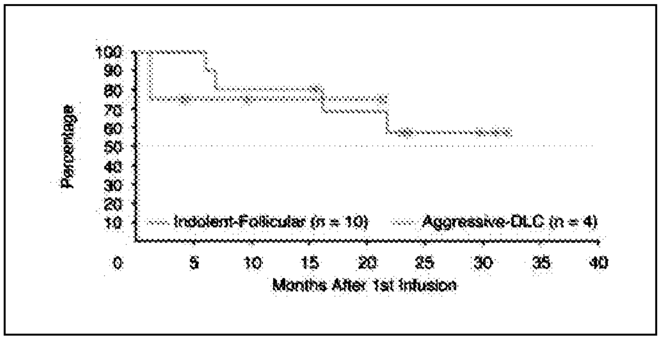

Fig 2. Kaplan-Meier curves of duration of response for patients with indolent-follicular and aggressive diffuse large B-cell lymphoma (DLBCL).

DISCUSSION

Therapy with rituximab is used widely in the treatment of indolent and aggressive NHL. In a registration trial that lead to initial approval, rituximab 375 mg/m^2 per week for 4 weeks in 166 patients with relapsed or refractory indolent NHL showed an OR rate of 48% (6% had CRs) and a median TTP of 13 months for responders.[10] In the follicular subset, response rates were higher (60%). In patients with aggressive NHL subtypes, rituximab therapy has an approximately 30% overall response rate, with a median of 8 months time to disease progression for responders.[18] AEs after single-agent rituximab treatment are generally brief and are usually related to the first infusion, but can include grade 3 and 4 toxicities.[10] With these encouraging results, the optimal use of rituximab in the treatment of indolent and aggressive NHL remains under active investigation, particularly through the use of maintenance therapy schedules.[19] Furthermore, there are continuing efforts to improve on the success of rituximab treatments for B-cell malignancies, including combinations with other biologic agents such as interleukin-2,[20,21] chemotherapy-rituximab regimens (eg, cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab),[7,22-25] and the development of second-generation CD20 MAbs.[26-28] This report describes the first clinical trial to evaluate a combination antibody therapeutic regimen targeting two distinct B cell-specific targets (CD20 and CD22).

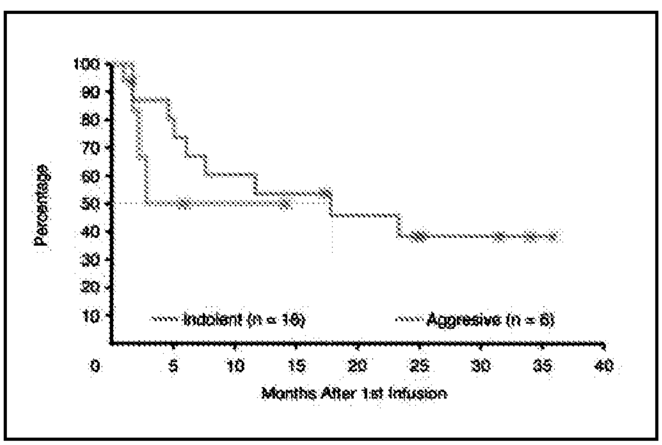

Fig 3. Kaplan-Meier curves of time to progression for all assessable patients (responders and nonresponders) with indolent and aggressive non-Hodgkin's lymphoma.

The hypothesis leading to this study was that antibodies directed against other B-cell antigens, and with potentially different mechanisms of action, might overcome any initial intrinsic resistance to rituximab, and could possibly also prove to be additive or synergistic when combined with rituximab. The candidate target chosen was CD22, a 135-kd transmembrane glycoprotein that is a B-lymphocyte–restricted member of the immunoglobulin superfamily, and a member of the sialoglycoprotein family of adhesion molecules that regulate B-cell activation and the interaction of B cells with T cells and antigen-presenting cells.[29,30] CD22 is detected on more than 85% of B-cell NHL.[13] Furthermore, preclinical data suggested that CD22 is an attractive target for B-cell–based therapy because of its restricted expression and potential enhancement of effects from an anti-CD20 therapy.[27]

The initial step in this approach included the development and characterization of a mouse monoclonal antibody (mLL2, formerly called EPB-2) that specifically binds to cluster C of human CD22.[31] Epratuzumab is a complementarity-determining region–grafted, humanized, IgG1 κ-engineered version of this murine MAb that is potentially more appropriate for repeated clinical use because less murine protein is present than in a chimeric MAb. Indeed, when naked and radiolabeled forms of epratuzumab were administered repeatedly to NHL patients, it was well tolerated and had virtually no immunogenicity.[12,13,32]

In vitro immunohistologic evaluations demonstrated an overlapping expression of CD20 and CD22 in samples of B-NHL.[33,34] Mechanistically, epratuzumab has not been found to cause B-cell killing by apoptosis or complement-mediated cytotoxicity, but has shown modest antibody-dependent cellular cytotoxicity when tested on NHL cell lines.[35] In contrast, all three mechanisms of action have been reported for rituximab,[36] as well as for other recently developed human CD20 MAb.[26,27] The mechanism of action of epratuzumab may in part be related to its rapid internalization after its binding to CD22-expressing lymphoma cells.[37]

We demonstrated single-agent activity of epratuzumab in phase I/II, single-center, open-label, dose-escalation studies in patients with NHL who had experienced disease relapse after conventional chemotherapy or rituximab treatment. In these studies, 55 patients with indolent NHL and 56 patients with aggressive NHL received once-weekly epratuzumab 120, 240, 360, 480, 600, and 1,000 mg/m^2 administered for 4 consecutive weeks.[12,13] In addition, 11 patients tolerated two treatment cycles, whereas one patient

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underwent three treatment courses.[38] Dose-limiting toxicity was not encountered in the initial dose escalation, and the study was expanded following additional experience at intermediate dose levels to include more patients receiving the 360 mg/m^2/wk dosing in the phase II arm. At this dose, six of 14 patients (43%) with follicular NHL and two of 13 patients (15%) with DLBCL achieved an objective response,[12,13] and several CRs were noted. Given that both anti-CD22 and anti-CD20 have clinical activity, through binding to different targets, evaluation of a combination regimen is clearly of interest.

In vitro studies also supported the potential improved efficacy of this antibody combination because of the observation that rituximab therapy could upregulate the expression of CD22.[33,35] Furthermore, murine experiments with human NHL xenografts showed that epratuzumab in combination with rituximab or with another CD20 MAb, hA20, may be more efficacious than either monotherapy.[27] We speculate that rapid internalization of CD22, especially after binding with epratuzumab, may result in activation of nonreceptor tyrosine kinases associated with phosphorylation of the cytoplasmic tail of CD22, as well as negative regulation of the B-cell antigen receptor, which could increase the antilymphoma effects of anti-CD20 agents.[33,35]

In this pilot trial, toxicity to the combination antibody regimen was similar in nature and degree to that previously reported with rituximab monotherapy. Though this is a small study, the OR rates were 63% and 67% with this outpatient, four-dose course of therapy of rituximab in combination with epratuzumab for patients with relapsed indolent and aggressive (DLBCL) NHL, respectively. It is important to note, however, that most (13 of 16) of the indolent NHL patients had a high-risk FLIPI score at study entry, although they also demonstrated some favorable prognostic features (median, one prior treatment regimen; median, 2.5 years since last therapy; rituximab naive). The aggressive NHL patients were more heavily pretreated (median, three prior treatment regimens), although their outcomes with the immediate prior therapy suggest that they may be characterized as more commonly having relapsed rather than refractory disease. With these caveats, the high CR rate in patients with recurrent NHL is noteworthy. Most of the responses were CR/CRu (56% for indolent/follicular NHL and 42% for DLBCL), which is uncommon for a well-tolerated biologic agent regimen in the setting of relapsed lymphoma.

Although patient characteristics differ across studies, it is encouraging that these CR rates are higher than the CR rate reported previously for rituximab alone in comparable dosing schedules.[10] Although one should be cautious in making any comparisons, Witzig et al[16] treated a cohort of 70 patients (83% with recurrent follicular lymphoma) using single-agent rituximab 375 mg/m^2 weekly for 4 weeks) in a study that also prospectively used the current International Workshop NHL response criteria. These investigators reported a CR rate of 16% and a CRu rate of 4%.[39] Preliminary results of two follow-up multicenter studies of epratuzumab plus rituximab also support the idea that some patients with B-cell NHL may demonstrate improved outcomes with a combination antibody approach.[40,41]

Although followup and further analysis are ongoing, initial results from these studies suggest that subsets of patients (including small lymphocytic lymphoma, DLBCL, and some follicular types) might benefit from the addition of epratuzumab to rituximab. However definitive conclusions cannot yet be made given the heterogeneous patient populations (histologies and prior therapies), short follow-up, limited subgroup numbers, and lack of a control (single antibody) comparison group. One could also speculate that there may be tumor types, based on antigen expression patterns, that could particularly benefit from combination antibody therapy. These groups might be identified through larger followup studies. Other approaches have been evaluated for their potential to augment the activity of rituximab. These include the addition of chemotherapy, the coadministration of immunostimulatory or pro-apoptotic agents, and the incorporation of a radioactive isotope.[21,23,42-44] Although efficacy data have shown benefits in some cases, these approaches may be limited by toxicities (including cytopenias, constitutional symptoms) and patient selection restrictions (eg, degree of bone marrow involvement).[40,44] The incorporation of a second active antibody without clinically meaningful additional toxicity, as in this report, is a particularly attractive strategy from the standpoint of limiting toxicity to patients.

The high CR rate and excellent tolerability of the combination of the CD20 and CD22 antibodies used in this study suggest a number of new possibilities for the treatment of indolent (follicular) NHL; this combination therapy should be tested in a large randomized study of patients with recurrent/refractory indolent NHL (to compare single-agent with combination therapy). The encouraging initial results observed for the combination in a small number of patients with DLBCL also suggest that this should be expanded in a cohort of patients with this aggressive form of NHL. If confirmed, this well-tolerated combination therapy may represent an acceptable alternative for patients with DLBCL who may have difficulty tolerating intensive chemotherapy, or in those who either may not be candidates for high-dose chemotherapy with peripheral stem-cell support or have experienced disease relapse after it.[43] A pilot study of cyclophosphamide, doxorubicin, vincristine, and prednisone plus epratuzumab and rituximab in first-line therapy for DLBCL has shown promising results,[45] and a larger multicenter study is under development.

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Characterization of a New Humanized Anti-CD20 Monoclonal Antibody, IMMU-106, and Its Use in Combination with the Humanized Anti-CD22 Antibody, Epratuzumab, for the Therapy of Non-Hodgkin's Lymphoma

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ABSTRACT

Purpose: **A new humanized anti-CD20 monoclonal antibody (MAb), IMMU-106, was evaluated to elucidate its action as an antilymphoma therapeutic, as a single agent, and in combination with the anti-CD22 MAb, epratuzumab.**

Experimental Design: **Antiproliferative effects, apoptotic effects, and the ability of IMMU-106 to mediate complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity on a panel of non-Hodgkin's lymphoma (NHL) cell lines were compared with the chimeric anti-CD20 MAb, rituximab, and evaluated in light of the various levels of antigen expression by the cell lines. *In vivo* therapy studies were performed in SCID mice bearing disseminated Raji lymphoma.**

Results: **The mechanisms of cytotoxicity of IMMU-106 were found to be similar to rituximab, and include direct apoptosis, antibody-dependent cellular cytotoxicity, and complement-mediated cytotoxicity. IMMU-106 was also found to be very similar to rituximab in terms of antigen-binding specificity, binding avidity, and dissociation constant. Treatment of Raji-bearing SCID mice with IMMU-106 yielded median survival increases of up to 4.2-fold compared with control mice. Survival in mice treated with IMMU-106 plus epratuzumab was compared with IMMU-106 treatment alone. Although the combined treatment did not improve median survival, an increased proportion of long-term survivors was observed. An enhanced antiproliferative effect was also observed *in vitro* in SU-DHL-6 cells when IMMU-106 was combined with epratuzumab. These findings are consistent with the up-regulation of CD22 expression observed after pretreatment of NHL cells *in vitro* with CD20 MAb (IMMU-106).**

Conclusions: **It is expected that in humans IMMU-106 should be at least as effective as rituximab and, due to its human framework construction, it may exhibit different pharmacokinetic, toxicity, and therapy profiles. In addition, it may be possible to enhance efficacy by combination therapy comprised of anti-CD20 and other B-cell lineage targeting MAbs, such as epratuzumab. The current results emphasize that *in vitro* as well as *in vivo* studies with many of the NHL cell lines were generally predictive of the known activity of anti-CD20 MAbs in NHL patients, as well as the enhanced efficacy of epratuzumab combined with rituximab observed in early clinical trials.**

INTRODUCTION

Pan-B-cell monoclonal antibodies (MAbs) have been demonstrated to be effective antilymphoma agents (1). Comparison of the relative merits of various anti-B-cell MAbs for therapy of B-cell malignancies has delineated the importance of several parameters in determining the ultimate efficacy of these agents, such as antigen density and the ability to induce complement-mediated cytotoxicity (CMC; Refs. 2–4), antibody-dependent cytotoxicity (ADCC; Ref. 5), and/or direct induction of apoptosis (6–8). It is likely that, depending on the system, more than one of these mechanisms plays a role in the effectiveness of a MAbs. It is also clear, however, that not all of the parameters have been elucidated, especially with regards to the properties that define which MAb would be a likely best choice across non-Hodgkin's lymphoma (NHL) subtypes and for individual cases within a subtype, as well as how to augment the efficacy of naked MAbs by combination with other treatment modalities.

The chimeric anti-CD20 antibody, rituximab (Rituxan; Genentech, South San Francisco, CA; IDEC, San Diego, CA), has been approved for the treatment of relapsed/refractory low-grade B-cell non-Hodgkin's lymphoma (9). Whereas rituximab is effective, only ~50% of patients respond when given 375 mg/m^2 weekly for 4 weeks (9). The median time to progression in responders is ~13 months (9), and ~60% of initial responders do not respond to retreatment (10). Because of these limitations, as well as the long initial infusion time necessary for administration of rituximab and the occurrence of infusion-related toxicities (11), there is an ongoing effort for improvement in this treatment modality. Other antibodies under investigation as potential therapeutic agents for NHL include anti-CD19, -CD22, -CD52, -CD74, -CD80, and -HLA-DR MAbs (12–19). As with rituximab, it is unlikely that these MAbs will be curative as single agents. Combination therapy,

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for example, with chemotherapy or of multiple MAbs targeting antigens with different signaling pathways may be necessary. The rational design of combination MAb therapy will depend on knowledge of the precise mechanism of action of the MAbs, with improved results expected when agents to distinct targets, which function by nonoverlapping mechanisms, are combined. It is also conceivable that binding to one antigen target may affect the binding to, or expression of, another.

In this report, a new humanized anti-CD20 MAb, IMMU-106, was evaluated to elucidate its action as an antilymphoma therapeutic. The humanized anti-CD20 MAb, IMMU-106 (also known as hA20; Immunomedics, Inc., Morris Plains, NJ), was generated using the same human IgG framework as epratuzumab (Immunomedics, Inc.), a CDR-grafted (humanized) MAb directed against CD22 (20). Antiproliferative effects, apoptotic effects, and the ability of IMMU-106 to mediate complement-dependent cell lysis and ADCC of NHL cell lines are compared with the chimeric anti-CD20 MAb and rituximab, and evaluated in light of the various levels of antigen expression by the cell lines. The expression of CD20 and CD22 by NHL cells in culture was also examined after pretreatment with either CD22 or CD20 MAbs. The ability of the MAbs to prolong survival in an animal model of NHL is also demonstrated. In addition, enhancement of the *in vitro* and *in vivo* antitumor effects of the anti-CD20 MAb, IMMU-106, is shown when given in combination with epratuzumab.

MATERIALS AND METHODS

Cells

The Burkitt lymphoma lines, Daudi, Raji, and Ramos, were purchased from the American Type Culture Collection (Manassas, VA). Non-Burkitt lymphoma cell lines were obtained as follows. RL and SU-DHL-6, which contain the chromosomal translocation t(14;18), were obtained from Dr. John Gribben (Dana-Farber Cancer Institute, Boston, MA) and Dr. Alan Epstein (University of Southern California, Los Angeles, CA), respectively. Cell lines SU-DHL-4, SU-DHL-10, and Karpas422 were provided by Dr. Myron Czuczman (Roswell Park Cancer Institute, Buffalo, NY), and WSU-FSCCL and DoHH2 cell lines were obtained from Dr. Mitchell Smith (Fox Chase Cancer Center, Philadelphia, PA). The cells were grown as suspension cultures in DMEM (Life Technologies, Inc. Gaithersburg, MD), supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM; complete media).

Antibodies

Development of hLL2, the humanized anti-CD22 MAb, now referred to as epratuzumab, has been described previously (20, 21). Similar procedures were adopted to develop the humanized anti-CD20 MAb, designated as IMMU-106, or hA20. Briefly, the V_κ and V_H genes of the parent anti-CD20 MAb were first cloned from the hybridoma cells by reverse transcription-PCR, and the complementary determining region sequences were elucidated by DNA sequencing, as described (22). The V genes of complementary determining region-grafted (or humanized) anti-CD20 MAbs were then designed and engineered. The same human framework regions (FRs) used for derivation of epratuzumab were applied, *i.e.,* the FR1, 2, and 3 of EU and the FR4 of NEWM heavy chain served as the scaffold for V_H, and the REI FRs as the scaffold for V_κ. IMMU-106 was expressed in Sp2/0-Ag14 cells (American Type Culture Collection). A high-level IMMU-106-producing clone was developed as described (21). Both epratuzumab and IMMU-106 were produced in bioreactors and purified by a combination of affinity chromatography on Protein A columns and gel filtration on SE columns under GMP compliance.

Other MAbs used in the studies were rituximab, purchased from IDEC Pharmaceuticals Corp. (San Diego, CA), and hMN-14, or labetuzumab (humanized anticarcinoembryonic antigen IgG_1), provided by Immunomedics, Inc. The construction and characterization of hMN-14, used here as a negative isotype control, have been described previously (23).

Cell Surface Antigen-Binding Assays

A competitive binding assay was used to evaluate the antigen-binding specificity of the anti-CD20 MAbs. A constant amount (100,000 cpm; ~10 μCi/μg) of ^{125}I-labeled rituximab was incubated with Raji cells in the presence of various concentrations (0.2–700 nM) of IMMU-106 or rituximab at 4°C for 1–2 h. Unbound MAbs were removed by washing the cells in PBS. The radioactivity associated with cells was determined after washing. The maximum number of binding sites per cell and the apparent antigen-binding affinity constant of the anti-CD20 MAbs were determined by direct cell surface saturation binding of the radiolabeled MAbs and Scatchard plot analysis, as described by Trucco *et al.* (24), and Lindmo *et al.* (25). MAbs were labeled with ^{125}I by the chloramine-T method (26). The data shown are specific binding. Each experiment was done with two sets of cells: (*a*) cells preincubated with respective cold MAb to block all of the binding sites; and (*b*) cells preincubated with medium. After preincubation, the cells were aliquoted, and radiolabeled MAb at various concentrations was added. The counts from set 1 were considered as nonspecific binding and that from set 2 total binding. Specific binding is total binding − nonspecific binding.

Flow Cytometric Assays

Immunophenotyping. Indirect immunofluorescence assays were performed with the panel of cell lines described above, using FITC-goat antimouse IgG (Tago, Inc., Burlingame, CA) essentially as described previously (27) and analyzed by flow cytometry using a FACSCalibur (Becton Dickinson, San Jose, CA).

Analysis of Apoptosis. Flow cytometric analysis of cellular DNA was performed after propidium iodide staining (6, 28). NHL cells were placed in 24-well plates (5×10^5 cells/well) and subsequently treated with MAbs (5 μg/ml). Three wells were prepared with each MAb to study the effects of cross-linking with goat antimouse or goat antihuman second antibodies. After a 20-min incubation with the primary MAbs (37°C; 5% CO_2), $F(ab')_2$ goat antimouse IgG Fcγ-specific second antibody (The Jackson Laboratory, West Grove, PA) was added to one well from each primary MAb to adjust the second antibody concentration to 20 μg/ml. $F(ab')_2$ goat antihuman IgG Fcγ-specific (The Jackson Laboratory) was similarly added to

the second well from each primary MAb, and the volume of the third set was equalized by addition of medium. After a 48-h incubation (37°C; 5% CO_2), cells were transferred to test tubes, washed with PBS, and then resuspended in hypotonic propidium iodide solution (50 mg/ml propidium iodide in 0.1% sodium citrate; 0.1% Triton X-100). Samples were analyzed by flow cytometry using a FACSCalibur. Percentage of apoptotic cells was defined as the percentage of cells with DNA staining before G_1/G_0 peak (hypodiploid).

Up-Regulation of Antigen Expression. To assess the effects of preincubation of NHL cells with IMMU-106 or epratuzumab, cells were stained with FITC-labeled anti-CD20 and -CD22 MAbs after overnight incubation with the unlabeled MAbs. Briefly, 1×10^6 cells were incubated in 2.0 ml of complete medium, or complete medium containing 10 µg/ml of IMMU-106 or epratuzumab, in triplicate, in 24-well plates. After a 17-h incubation (37°C; 5% CO_2), cells were transferred to test tubes, washed with PBS and 1% BSA, and then resuspended in 350 µl PBS and 1% BSA. An aliquot (100 µl) from each incubation mixture was incubated with either FITC-CD8 (Becton Dickinson: anti-Leu-2a-FITC; molar ratio FITC:protein = 4:9) as a negative control, FITC-anti-CD20 (Beckman Coulter: B1-FITC; molar ratio FITC:protein = 5:10), or FITC-anti-CD22 (Caltag: RFB4-FITC; molar ratio FITC:protein = 6.07), according to the manufacturer's directions, for 30 min, then washed with PBS and 1% BSA, resuspended in 1% formalin, and analyzed by flow cytometry with the Becton Dickinson FACSCalibur.

Cytotoxicity Assays

Standard ^{51}Cr release assays were performed for the measurement of ADCC and CMC essentially as described (29). All of the assays were performed in triplicate. Blood specimens used in these studies were collected under a protocol approved by the Institutional Review Board. Normal human serum complement was purchased from Quidel Corporation (San Diego, CA). For the CMC assay, 25 µl of 1:5 dilution was added, followed by a 3-h incubation. For the ADCC, E:T cell ratios of ~50:1 were used, and incubations were for 4 h. All of the blood donors gave voluntary, written informed consent.

Percentage of specific lysis was calculated according to the following formula:

% lysis = [^{51}Cr release from experimental sample − spontaneous^{51}Cr release]/[^{51}Cr release from maximum release − spontaneous ^{51}Cr] × 100.

In Vitro Cell Proliferation Assays

Effects of MAbs, with or without Second Antibody Cross-Linking on [^{3}H]Thymidine Uptake. MAb effects on cell growth were determined by assessing [^{3}H]thymidine incorporation in the NHL cell lines with and without the presence of a cross-linking second antibody, essentially as described by Shan *et al.* (6). Second antibodies used for evaluating the effects of cross-linking were $F(ab')_2$ goat antimouse IgG Fcγ-specific or $F(ab')_2$ goat antihuman IgG Fcγ-specific (The Jackson Laboratory). All of the tests were performed in triplicate.

In Vivo Effects of Naked MAbs on SCID Mice Bearing Disseminated Raji

Mice were injected i.v. with 1–2.5 × 10^6 Raji cells on day 0. Administration of MAbs was initiated 1 day after injection of tumor cells according to dose schedules described for each experiment. Mice were examined daily for signs of distress or hind-leg paralysis and weighed weekly. Paralysis of the hind legs or weight loss of >25% was used as the survival end point. Animals were euthanized at these end points. Animal studies were performed under protocols approved by the Institutional Animal Care and Use Committee.

RESULTS

Antigen-Binding Characteristics of IMMU-106. IMMU-106 was designed to have human IgG1/κ constant regions and the same human V FRs as the humanized anti-CD22 antibody, epratuzumab. The antigen-binding specificity and affinity of IMMU-106 were evaluated by cell-surface competitive and direct saturation-binding assays, and compared with rituximab, a human-mouse chimeric anti-CD20 MAb. In the competitive binding assay, various concentrations of IMMU-106 or rituximab were used to compete with radioiodinated rituximab for the binding to Raji human NHL cells. The results shown in Fig. 1*A* confirmed that IMMU-106 has the same antigen-binding specificity as rituximab and the apparent binding avidities are comparable between these MAbs. This was additionally confirmed by direct cell surface saturation binding and Scatchard plot analysis to measure the dissociation constant of IMMU-106. As shown in Fig. 1*B,* the apparent dissociation constant values for IMMU-106 and rituximab were virtually the same, calculated to be 3.6 ± 0.6 and 3.1 ± 0.4 nM, respectively. Similar results were obtained on Daudi cells (data not shown).

Antigen Expression of Cultured Lymphoma Cell Lines and Normal Peripheral Blood Lymphocytes. Flow cytometry analysis was performed using indirect immunofluorescent staining to show that IMMU-106 binds to a panel of cultured B-cell lymphomas. As shown in Table 1, the MAb binds to all of the tested cell lines, but the level of fluorescence staining varied between the cell lines. IMMU-106 behaves similarly to rituximab, staining the Burkitt cell lines, Raji, Ramos, and Daudi, and the non-Burkitt (follicular and diffuse large B-cell lymphoma) lines, SU-DHL-6, RL, and DoHH2, with high intensity. Four other NHL lines, WSU-FSCCL, Karpas422, SU-DHL-4, and SU-DHL-10, exhibited lower levels of MAb staining. SU-DHL-6 had the highest staining intensity, followed by Raji. Examples of histograms representing 3 levels of staining intensity are shown in Fig. 2 for the SU-DHL-4, Raji, and SU-DHL-6 cell lines. The mean fluorescence intensity of IMMU-106 staining averaged 78% (range, 56–95%) of the rituximab levels. It is possible that this difference reflects a difference in the binding of the FITC-labeled second antibody (goat antihuman IgG) to the chimeric (rituximab) and humanized (IMMU-106) MAbs. This is supported by the observation that a human-mouse chimeric version of IMMU-106 yielded equivalent results to rituximab (mean percentage of rituximab value = 102%; range, 90–116%; data not shown).

MAb binding to normal human peripheral blood leukocytes also was assessed. Lymphocytes, monocytes, and granulocytes

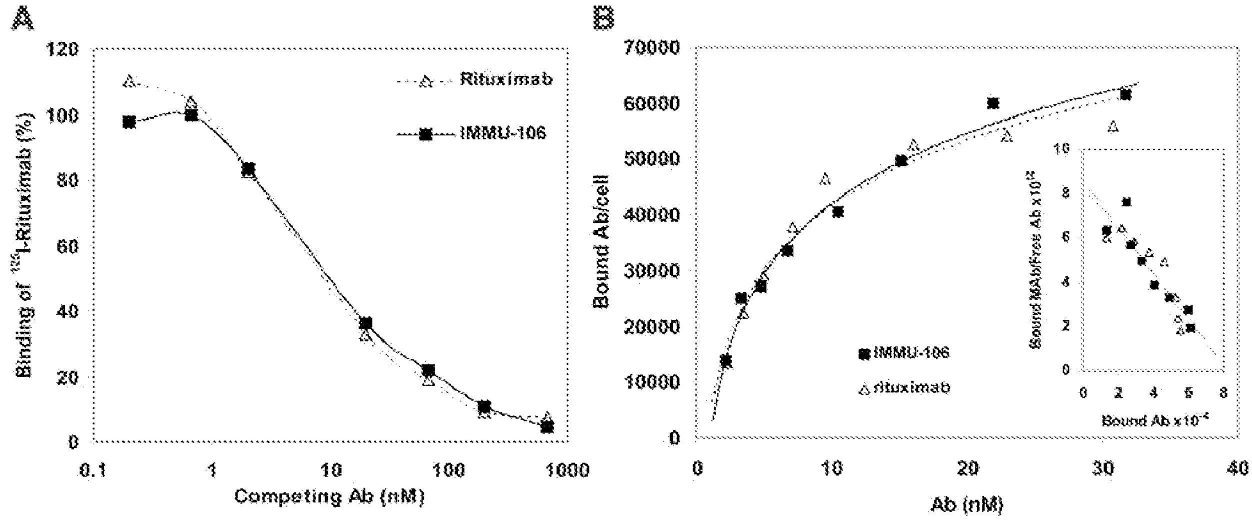

Fig. 1 Binding characteristics of IMMU-106. *A,* competitive binding assay. A cell surface competitive binding assay was performed to compare the binding activity of IMMU-106 with that of rituximab. Varying concentrations of IMMU-106 (■) or rituximab (△) were mixed with a constant amount of ^{125}I-rituximab and incubated with Raji cells at 4°C for 1 h. The cells were washed to remove unbound monoclonal antibodies and counted for the bound residual radioactivity. *B,* direct cell surface saturation binding and Scatchard plot analysis. IMMU-106, ■; rituximab, △; *Ab,* antibody.

were incubated with the anti-CD20 MAbs, followed by staining with FITC-labeled goat antihuman second antibody. Flow cytometry analysis indicated that staining of normal peripheral blood lymphocytes was similar for IMMU-106 and rituximab (Table 2). Positive staining was ~9% above the background with the anti-CD20 MAbs, which is within the normal range for percentage of B cells. Monocytes and granulocytes were negative.

Effects of Naked MAbs on Proliferation of NHL Cell Lines. Growth inhibition by the anti-B-cell MAbs was evaluated by *in vitro* proliferation assays in the NHL cell lines. Cells were cultured with the MAbs in solution with or without a second MAb for cross-linking, to mimic the role of effector cells *in vivo*. Proliferation was assessed by measuring the uptake of [^{3}H]thymidine. Controls included rituximab, no first MAb, and a negative control MAb, hMN-14. In all of the B-cell lines studied, specific inhibition was seen with the anti-CD20 MAbs, but the level of inhibition varied between the cell lines. As shown in Fig. 3, anti-CD20 MAbs yielded specific inhibition of proliferation in the Burkitt and non-Burkitt lymphoma cell lines. However, inhibition of proliferation was not directly related to antigen density. For example, CD20 expression is greater in Raji than Daudi, yet inhibition of proliferation of Daudi cells by anti-CD20 MAbs was greater than that of Raji cells. In the Raji experiment, inhibition of proliferation by the anti-CD20 MAbs was ~20% and with cross-linking ~40%, compared with Daudi in which >60% inhibition was seen with cross-linked anti-CD20 MAbs, IMMU-106, and rituximab. Among the non-Burkitt lymphoma cell lines, SU-DHL-6 was markedly more sensitive to antiproliferative effects of the MAbs than RL, SU-DHL-4, and SU-DHL-10, as well as the Burkitt lines. In the absence of cross-linking, IMMU-106 and rituximab yielded ~88% inhibition of proliferation of SU-DHL-6 cells, and with cross-linking specific inhibition of proliferation increased to 98%. Results with these cell lines again indicate that inhibition of proliferation is not directly related to antigen density. Whereas CD20 expression is in the order SU-DHL-6 > RL > Raji > Daudi, sensitivity of proliferation to anti-CD20 MAbs is in the order SU-DHL-6 > Daudi > Raji > RL. SU-DHL-4 and SU-DHL-10 express low levels of CD20 and are relatively insensitive to the anti-CD20 MAbs (data not shown).

Mechanistic Studies. Apoptosis, ADCC, and CMC were evaluated using a panel of B-cell lymphoma cell lines.

Induction of Apoptosis by Naked MAbs in NHL Cell Lines. Induction of apoptosis was evaluated by flow cytometry assays on the B-cell line panel. Cells were cultured with the MAbs for 48 h with or without a second MAb for cross-linking, followed by DNA staining with propidium iodide. Cells were analyzed by flow cytometry, and positive florescence below the G_1 region represents DNA fragmentation and is a measure of apoptosis. Controls included rituximab, no first MAb, and the isotype negative control MAb, hMN-14. Results with SU-DHL-6 cells are shown in Fig. 4. In all of the B-cell lines studied, specific induction of apoptosis was seen with the anti-

Table 1 Antigen expression: indirect flow cytometry assay (geometric mean fluorescence)

	hMN-14	Rituximab	IMMU 106
Burkitt's			
Daudi	5.9	252.9	222.6
Raji	2.2	384.7	268.4
Ramos	1.1	119.5	82.6
Non-Burkitt's			
DoHH2	4.8	45.6	41.9
Karpas422	8.3	12.2	11.6
RL	3.1	158.9	130.8
SU-DHL-4	2.5	46.8	26.3
SU-DHL-6	1.6	599.5	439.2
SU-DHL-10	2.5	35.7	26.5
WSU-FSCCL	3.0	36.4	28.5

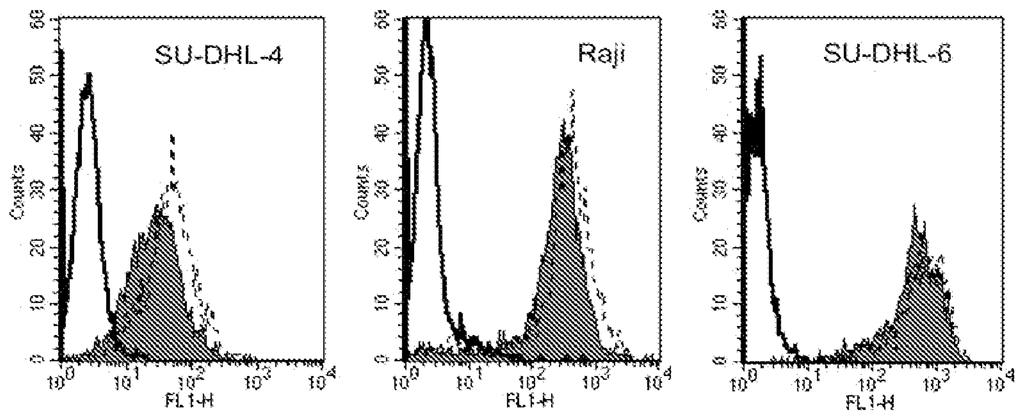

Fig. 2 Antigen expression on non-Hodgkin's lymphoma cell lines. Flow cytometry histograms are shown for three of the cell lines listed in Table 1. *Open solid line,* hMN-14; *filled solid line,* hA20; *dashed line,* rituximab.

Table 2 MAb[a] binding to peripheral blood lymphocytes

	Mean fluorescence	Percent positive
No first MAb	13	6.7
hMN-14	19	10.1
Rituximab	34	15.4
IMMU-106	30	15.6

[a] MAb, monoclonal antibody.

CD20 MAbs when an appropriate cross-linking agent was used. In the majority of cell lines apoptosis was not induced with any of the tested MAbs in the absence of cross-linking (Table 3). SU-DHL-6 is the exception; in this cell line the anti-CD20 MAbs also induced apoptosis without cross-linking.

ADCC and CMC. The ability of the anti-B-cell MAbs to induce ADCC and CMC was assayed using standard ^{51}Cr release assays and a homogeneous fluorometric lactate dehydrogenase release assay (Promega; data not shown). As measured by both methods, incubation of B-lymphoma cells with rituximab and IMMU-106 caused ADCC and CMC in the presence of human peripheral blood mononuclear cells or human complement, respectively (Figs. 5 and 6). Similar to the results observed in the proliferation and apoptosis evaluations, sensitivity of cell lines to ADCC varied, as noted by the *Y*-axis scales in Fig. 5. Levels of cytotoxicity were similar for rituximab and IMMU-106 in these studies.

***In Vivo* Effects of Naked MAbs on SCID Mice Bearing Disseminated Raji Lymphoma.** *In vivo* therapy studies were performed in SCID mice bearing systemic Raji tumors. Mice were injected i.v. with Raji cells on day-0. Fig. 7 shows a comparison of the anti-CD20 MAbs IMMU-106 and rituximab. MAbs were administered i.p. 5 times/week for 2 weeks, at 100 μg/injection, starting 1 day after injection of Raji cells, then twice weekly until day 36 of the study. Control mice received 100 μl of PBS, the MAb diluent, on each injection date. Control mice died of disseminated disease manifested with central nervous system paralysis, with a median survival time of 16.5 days

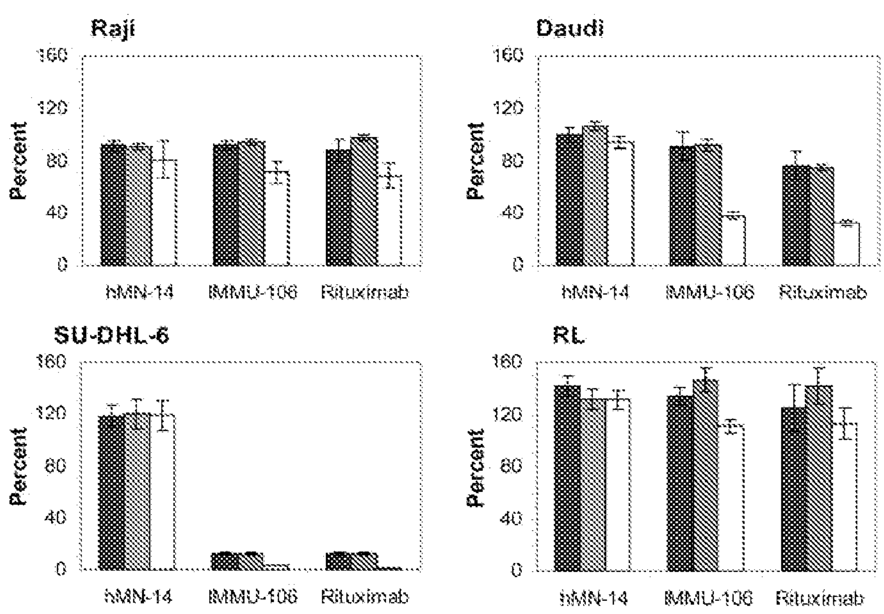

Fig. 3 Effects of anti-CD20 monoclonal antibodies (*MAbs*) on proliferation of non-Hodgkin's lymphoma cell lines. Antiproliferative effects of the anti-B-cell MAbs were assessed by measuring the uptake of [^{3}H]thymidine. Cells were cultured with the MAbs with or without a second antibody for cross-linking to mimic the role of effector cells *in vivo*. Results of four cell lines are shown; *bars,* ±SD. ■, no second Mab; ▒, antimouse; □, antihuman.

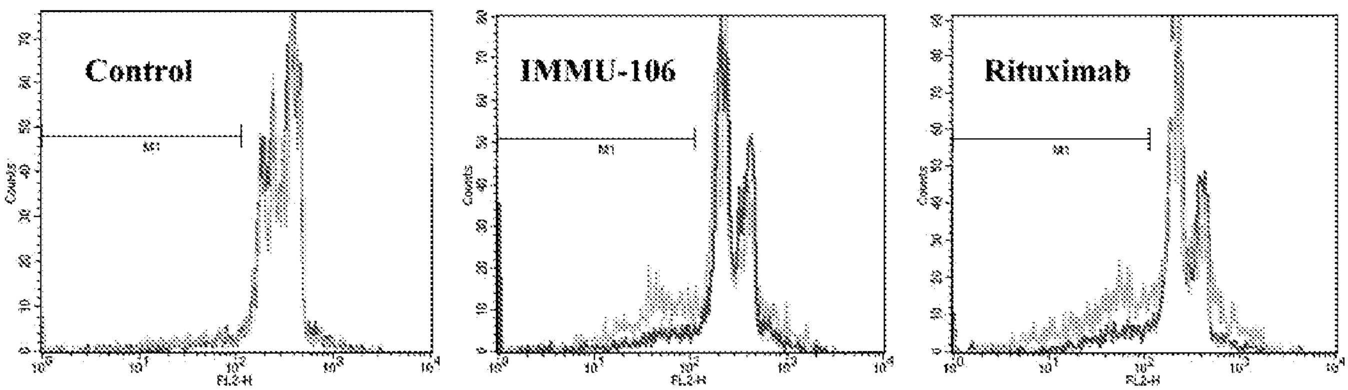

Fig. 4 Apoptotic effect of monoclonal antibodies on SU-DHL-6 cells. Induction of apoptosis was evaluated by flow cytometry determination of hypodiploid DNA on the B-cell line panel. Cells were cultured with the monoclonal antibodies without a second antibody (*purple line*), with goat antimouse IgG (*pink line*), or with goat antihuman IgG (*green line*), followed by DNA staining with propidium iodide. Results with SU-DHL-6 cells are shown. Hypodiploid DNA peaks corresponding to apoptotic nuclei were quantified in region M1.

after Raji tumor inoculation. Median survival in the treated groups was extended to 98 days for rituximab and 70 days for IMMU-106, statistically significant survival extensions in this model by log rank analysis ($P < 0.0001$). No statistical difference was observed between the effects of IMMU-106 and rituximab. These values represent median survival increases of 5.9-, and 4.2-fold for rituximab and IMMU-106, respectively, compared with control mice. Subsequent studies evaluated the importance of the Fc region for effective therapy by comparing the anti-CD20 MAbs, IMMU-106, and rituximab, and their $F(ab')_2$ fragments. The $F(ab')_2$ fragments were ineffective (data not shown), with identical median survival to control animals, confirming previous reports on the importance of Fc-mediated functions (CMC or ADCC).

Effects of Combining Anti-B-Cell MAbs. Combinations of MAbs recognizing distinct tumor-associated antigens can potentially enhance antitumor activity. To explore this possibility, the effects of combining IMMU-106 and epratuzumab were studied *in vitro* by evaluating effects on proliferation of cells in culture and *in vivo* in SCID mice bearing disseminated Raji tumors. As shown in Figs. 8 and 9, the combination of the two naked MAbs appears to be more effective than either agent alone.

Fig. 8 shows the results of an *in vitro* proliferation assay by [^{3}H]thymidine uptake. IMMU-106 alone caused a 53% inhibition of proliferation of SU-DHL-6 cells, and epratuzumab alone had no effect. The combination of the two agents increased inhibition of proliferation to 83% ($P < 0.001$, a significant difference from effect of IMMU-106 alone). This level of inhibition is similar to that obtained by cross-linking IMMU-106 with goat antihuman second antibody.

The survival curves shown in Fig. 9 represent combined data of two experiments comparing the effects of IMMU-106 given alone and in combination with epratuzumab. Each MAb was administered at 50 μg/injection, twice weekly, starting 1 day after tumor cell injection. In the combined MAb treatment group, each MAb was given twice weekly at 50 μg/injection. This dose is lower than that administered in the experiment shown in Fig. 7 and was selected to facilitate observation of improvements caused by the MAb combination. Median survival was 15 days in the untreated, isotype-matched control (hMN-14) and epratuzumab groups. IMMU-106 administered alone increased median survival to 25 days, and the combination of IMMU-106 and epratuzumab yielded a small increase in median survival; however, prolonged survival was observed in 30% of the mice. Day 35 was the time point at which the last animal reached the end point (hind-leg paralysis) in the IMMU-106-alone treatment group, whereas in the IMMU-106+epratuzumab group, 6 of 20 mice were still surviving at this time point. Survival in these 6 mice ranged from 43 to 72 days. Statistical significance of the effect was barely not reached *in vivo* (P = 0.0515 log rank test) for the difference between IMMU-106 and IMMU-106+epratuzumab.

Up-Regulation of CD22 by Anti-CD20. Mechanisms of enhancement of efficacy may include up-regulation of antigen levels as well as synergy between two different signaling pathways. Impact on receptor expression was examined by studying the CD22 and CD20 antigen density on cultured B-cell lines

Table 3 Apoptotic effect of anti-CD20 MAbs[a] as shown by propidium iodide staining (% hypodiploid DNA)

1st MAb/ 2nd MAb	SU-DHL-6			Daudi			RL		
	None	GAM	GAH	None	GAM	GAH	None	GAM	GAH
None	4.2	3.9	7.0	1.4	1.6	1.3	2.4	3.4	2.0
hMN-14	3.9	4.0	6.7	1.3	1.2	2.2	1.8	4.9	4.6
Rituximab	9.4	10.3	25.7	1.2	1.6	15.0	3.7	4.4	14.1
IMMU-106	10.6	9.3	29.5	1.5	1.5	14.6	3.4	2.7	25.1

[a] MAb, monoclonal antibody.

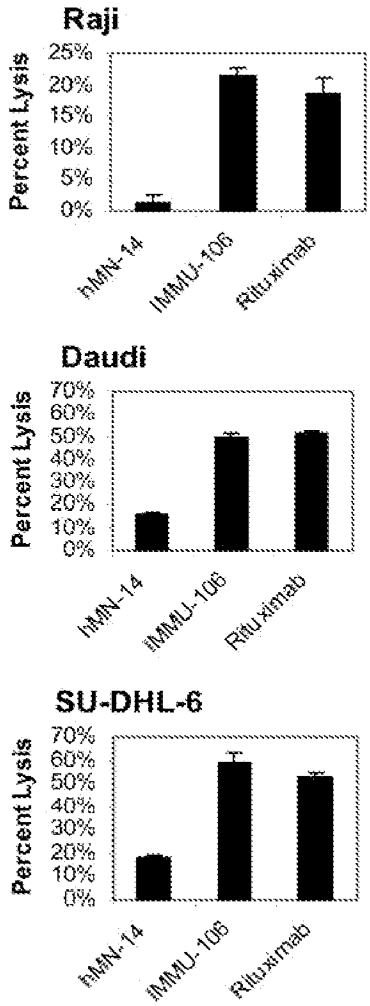

Fig. 5 Antibody-dependent cytotoxicity on non-Hodgkin's lymphoma cell lines. ^{51}Cr-labeled non-Hodgkin's lymphoma cells were incubated with anti-B-cell monoclonal antibodies in the presence of human peripheral blood mononuclear cells. The cells were incubated for 4 h at 37°C, followed by collection and counting of supernatants. Percentage of specific lysis of three cell lines is shown; *bars,* ±SD.

after incubation of the cells with epratuzumab or IMMU-106. Fig. 10 shows the flow cytometry histograms demonstrating that CD22 expression is up-regulated after overnight incubation with IMMU-106. As shown in Fig. 10, the histogram representing CD22 expression level after exposure to IMMU-106 is shifted to the right relative to the histogram representing CD22 expression with no prior exposure to IMMU-106. Mean fluorescent intensity increased from 21 to 28, an increase of 33%. Incubation of cells with epratuzumab did not increase the density of CD20 (data not shown).

DISCUSSION

Several issues must be considered to understand and try to improve upon the success of antibody-based treatments for NHL. The work reported herein addresses some of these issues. First, in an effort to improve upon the results obtained with rituximab, a humanized anti-CD20 MAb was developed by complementary determining region grafting. Rituximab is a murine-human chimeric MAb, in which the variable domains are derived from the murine anti-CD20 MAb, and the constant regions from human IgG1 heavy chain and human κ light chain. Although a chimeric antibody is less likely than a fully murine MAb to provoke an immune response, and elicitation of a human antichimeric antibody response has not posed a significant obstacle to the use of rituximab, it may be advantageous clinically to have a more fully human version, especially if repeated injections may be desired in patients, *e.g.,* for nonmalignant diseases, such as autoimmune diseases. Administration of humanized MAbs could possibly result in altered pharmacokinetic and toxicity profiles. A possible extension in serum half-life may permit extended dosing intervals and lead to reduced immunogenicity. Changes in pharmacokinetics and dosing regimens may affect the therapeutic response as well as toxicity. Although these benefits are theoretical at this time, remaining to be proven in clinical studies, such studies with

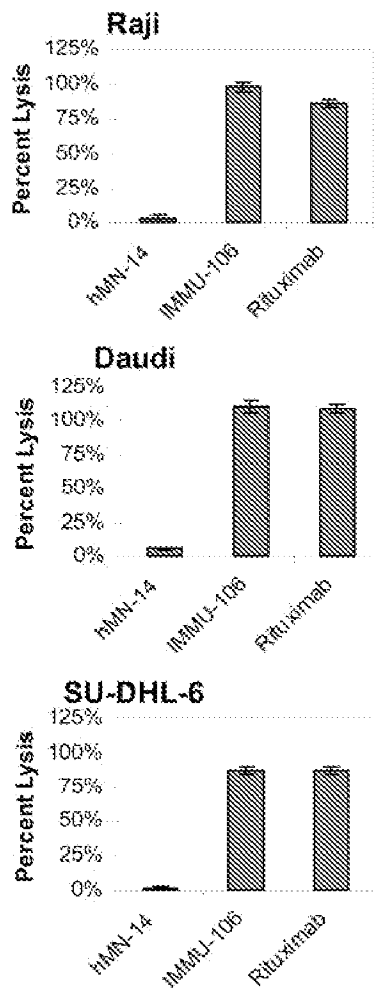

Fig. 6 Complement-mediated cytotoxicity on non-Hodgkin's lymphoma cell lines. ^{51}Cr-labeled non-Hodgkin's lymphoma cells were incubated with anti-B-cell monoclonal antibodies in the presence of human complement. The cells were incubated for 3 h at 37°C, followed by collection and counting of supernatants. Percentage of specific lysis of three cell lines is shown; *bars,* ±SD.

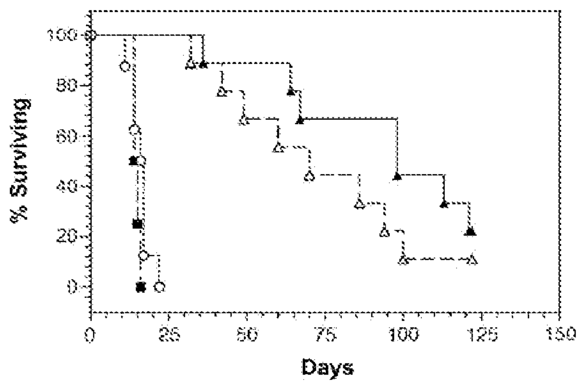

Fig. 7 Survival proportions in SCID mice bearing disseminated Raji cells; comparison of IMMU-106 and rituximab. Monoclonal antibodies were administered to SCID mice 5 times/week for 2 weeks, at 100 μg/injection, then twice weekly until day 36 of the study. Monoclonal antibody injections were initiated 1 day after i.v. injection of Raji cells. Each treatment group contained 10 mice. ○, untreated; –■–, hMN-14; –△–, IMMU-106; —▲—, Rituximab.

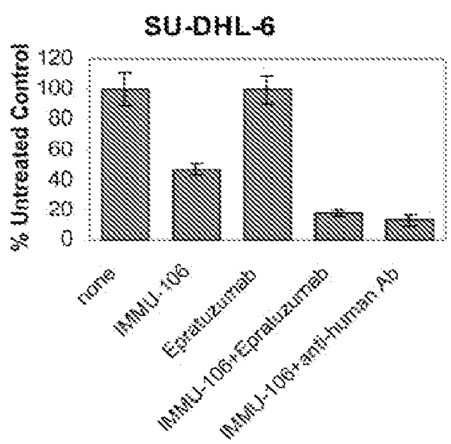

Fig. 8 Antiproliferative effects of the combination of IMMU-106 and epratuzumab by *in vitro* [^{3}H]thymidine uptake assay. SU-DHL-6 cells were cultured either without any added monoclonal antibody, or with IMMU-106, epratuzumab, the combination of IMMU-106 and epratuzumab, or the combination of IMMU-106 and goat antihuman IgG Fcγ-specific, for 48 h, followed by the addition of [^{3}H]thymidine and another 16-h incubation; *bars,* ±SD.

epratuzumab have demonstrated improved infusion properties, consisting of 30–60-min infusions (30) compared with infusion times of >4 h for rituximab (11). Epratuzumab administration has resulted in less infusion-related toxicity than has been evident with rituximab, and virtually no immune responses have been observed in patients given either the naked (30) or radioconjugated humanized epratuzumab, even when repeated, fractionated doses were administered (31). Although the different target antigen specificities of epratuzumab and rituximab may partly contribute to the different infusion characteristics, the different framework regions may also play an important role.

Second, we used a panel of cell lines to evaluate the ability of the MAbs to kill NHL cells. Cell lines were included as a variable because our experience has shown that various cell lines respond differently to immunotherapy. We observed differences in the ability of the MAbs to inhibit proliferation, as well as induce apoptosis, ADCC, and CMC. These results were not directly related to antigen density. This is consistent with the observations of others with anti-CD20 MAbs (32) as well as other anti-B-cell MAbs (16). Nagy *et al.* (16) reported a nonlinear correlation between killing efficiency with anti-HLA-DR MAbs and the level of HLA-DR antigen expression. Chan *et al.*

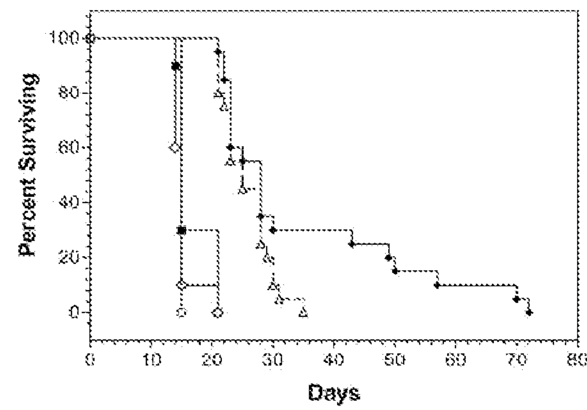

Fig. 9 Combination therapy IMMU-106 and epratuzumab in Raji-bearing SCID mice. Monoclonal antibodies were administered to SCID mice 2 times/week at 50 μg/injection. Monoclonal antibody injections were initiated 1 day after i.v. injection of Raji cells. Results shown include combined data from animals treated on two dates. The first included animals treated with hMN-14 (n = 10), IMMU-106 (n = 10), epratuzumab (n = 10), and IMMU-106+epratuzumab (n = 10). The second included animals treated with IMMU-106 (n = 10), IMMU-106+epratuzumab (n = 10), and control untreated mice (n = 9). ▲, IMMU-106; ◆, IMMU-106+Epratuzumab; ◇, Epratuzumab; ■, hMN-14; ○, untreated.

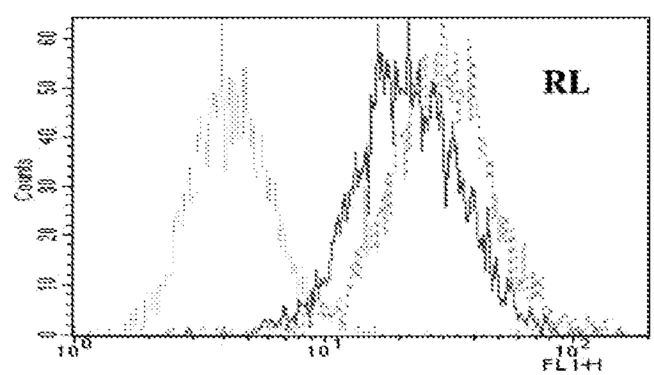

Fig. 10 Up-regulation of CD22 by anti-CD20 (IMMU-106) in RL cells. Cell lines were incubated ~17 h with a saturating concentration of IMMU-106 or no monoclonal antibody (MAb). The CD22 and CD20 expression was then measured by flow cytometry using FITC-RFB4, an anti-CD22 MAb that blocks epratuzumab binding, and FITC-B1, which blocks IMMU-106 binding. CD8 expression was also measured as a negative control (FITC-anti-Leu-2a). RL cells stained with FITC-RFB4 either without MAb pretreatment (*purple line*) or after pretreatment with IMMU-106 (*green line*) are shown in comparison to the control untreated cells stained with FITC-anti-Leu-2a (*red line*). FITC-B1 staining is not shown.

(32) observed differences in the effects of anti-CD20 MAbs on a range of cell lines by various apoptotic and clonogenic assays. By analyzing the differences between cell lines, these authors concluded that apoptosis through CD20 was dependent on the nature of MAb binding and correlated with the extent of homotypic cell adhesion induced. In addition, they concluded that the extent of apoptosis was independent of translocation to Triton X-100 insoluble rafts, and that CD20 can evoke apoptosis without involvement of mitochondria and caspases. Control of sensitivity to CD20-induced apoptosis remains unclear; it is likely that signaling or effector molecules are missing or improperly regulated in insensitive tumor cell lines. These observations highlight the importance of studying a number of cell lines before drawing a conclusion on clinical relevance, as well as demonstrating how correlating mechanistic assays with efficacy observations can shed light on innate and/or acquired resistance to antibody therapy.

Third, we examined the effects of combining anti-CD20 and anti-CD22 treatments. Epratuzumab, and the anti-CD20 MAbs IMMU-106 and rituximab, recognize distinct antigens and achieve their efficacy through different mechanisms. Anti-CD20 MAbs, including rituximab, have been shown to induce ADCC and CMC in tumor target cells (2–5, 33). In addition, evidence from *in vitro* studies, animal tumor models, and clinical trials suggest that the tumoricidal effect of naked anti-CD20 MAbs does not occur solely by these mechanisms. Antibody binding to CD20 has been shown to inhibit cell cycle progression after mitogen stimulation (34); inhibit B-lymphocyte differentiation (35); inhibit EBV and pokeweed mitogen-induced immunoglobulin secretion (34, 35); and generate a transmembrane signal that results in enhanced phosphorylation of the molecule (36), increase of tyrosine-kinase activity (37), and induction of c-myc oncogene expression (38). Shan *et al.* (6) demonstrated that extensive cross-linking of CD20 with murine anti-CD20 MAbs in the presence of either goat antimouse IgG or Fc receptor-expressing cells directly inhibits B-cell proliferation, induces nuclear DNA fragmentation, and leads to cell death by apoptosis. Apoptotic effects can be inhibited by chelation of intracellular or extracellular calcium ions. These results suggest that ligation of CD20 *in vivo* by MAbs in the presence of Fc receptor-expressing cells may initiate signal transduction events, increase calcium ion levels, and lead to apoptosis.

CD22 functions as an adhesion receptor for B cells, T cells, monocytes, neutrophils, and RBCs (39), and is involved in signal transduction, modulating B-cell antigen receptor-mediated signal transduction (40). Ligation of CD22 with MAbs that block the ligand-binding site triggers rapid tyrosine phosphorylation of CD22 and primary B-cell proliferation (41). In contrast to the binding of the anti-CD22 MAb, HB22.23, epratuzumab does not block the ligand-binding site on CD22 (42). However, ligation of CD22 with epratuzumab does cause rapid internalization of the MAb and also induces phosphorylation of the CD22 cytoplasmic tail (42). The rapid internalization of this MAb into NHL cells has facilitated its use for the delivery of toxins within the cells (43, 44). A key role for CD22 in B-cell function is also suggested by studies showing that CD22-deficient mice have a shorter life span, a reduced number of mature B cells, a chronic exaggerated antibody response to antigen, and development of elevated levels of autoantibodies (45). Many of these functions can be modulated by epratuzumab through CD22 phosphorylation and CD22 internalization (42).

In clinical studies epratuzumab has shown antitumor responses as an unlabeled agent (30) and in radioimmunotherapy applications (31, 46, 47). Unlabeled, epratuzumab has shown evidence of antitumor activity in patients with recurrent NHL, producing responses, including complete responses, in patients with follicular and diffuse large B-cell histologies. In a dose escalation study examining the safety, efficacy, and pharmacokinetics of epratuzumab in patients with recurrent indolent NHL, Leonard *et al.* (30) administered 120-1000 mg/m^2 over 30–60 min for four weekly treatments. These doses were well tolerated with no dose-limiting toxicities and had clinical activity. A 43% objective response rate was observed in follicular NHL patients receiving 360 mg/m^2/week, similar to results observed using rituximab. Greater than 95% of the infusions were completed in $\sim$1 h. The fact that epratuzumab does not activate complement and, thus, produces a less-dramatic depletion of B cells may contribute to the excellent infusion tolerability. In addition, these dosing regimens resulted in an extended half-life of 23 days, compared with $\sim$10 days with rituximab (30).

Thus, the combination of epratuzumab with an anti-CD20 MAb may be beneficial, yielding additive or synergistic activities. Preliminary findings in a murine model (48) and in patients (49) indicate that the combination of rituximab and epratuzumab is well tolerated and may result in improved antilymphoma activity *versus* the single agents. Clinically, the overall response rate of epratuzumab combined with rituximab in indolent, follicular NHL was reported to be similar to that of rituximab alone, but a higher complete response rate was found (49). Thus, it would seem that these two antigen targets overlap in terms of responsiveness in this tumor type, with the combination only improving the magnitude of the rituximab response in contrast to more patients responding when epratuzumab is added. This implies that the combination is synergistic in those patients having both target antigens and responding to either agent.

As reported here, we found that the combination of IMMU-106 and epratuzumab appears to be more effective than either MAb alone in the SU-DHL-6 tumor cell line. *In vitro*, the combination of these two MAbs increased inhibition of proliferation from 53% for IMMU-106 alone to 83% in cultured SU-DHL-6 cells. *In vivo*, in SCID mice bearing disseminated Raji cells, prolonged survival was observed in 30% of the mice given IMMU-106 and epratuzumab, compared with IMMU-106 monotherapy. These results are consistent with the clinical observations and may be explained partially by the up-regulation of CD22 expression after treatment of NHL cells with CD20 MAb, implying that higher antigen expression may contribute to more effective therapy. It is not clear why cross-linking of CD20 increases CD22 expression and not *vice versa*. Additive or synergistic results on signaling events initiated by the anti-CD20 and -CD22 MAbs may be the cause of the increased efficacy when the two agents are used in combination, although there may be little or no observed antiproliferative effects of the anti-CD22 MAb when given alone. Although there are many limitations to the use of xenografted NHL cell lines, especially because the SCID or nude mice do not have normal B cells that express the target human B-cell lineage antigens in question,

many of the observations made clinically can be reproduced in some of these models, such as the efficacy of naked CD20 MAbs as a monotherapy and in combination with epratuzumab shown in this article. The fact that the animal model reflects what has been seen in clinical studies encourages us to pursue the study of other variables in the preclinical setting, which would be impractical, if not impossible, to evaluate clinically.

In conclusion, the data shown here suggest that the mechanisms of cytotoxicity of IMMU-106, like rituximab, include direct apoptotic effects, as well as ADCC and complement-mediated cell lysis. It is expected that in humans, IMMU-106 should be at least as effective as rituximab and, due to its construction based on the framework of epratuzumab, it may exhibit different pharmacokinetic, toxicity, and therapy profiles. In addition, the results indicate that it may be possible to enhance efficacy by combination therapy comprised of anti-CD20 and other B-cell lineage targeting MAbs, such as epratuzumab, which supports current clinical studies of the combination of rituximab and epratuzumab in NHL therapy (49).

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